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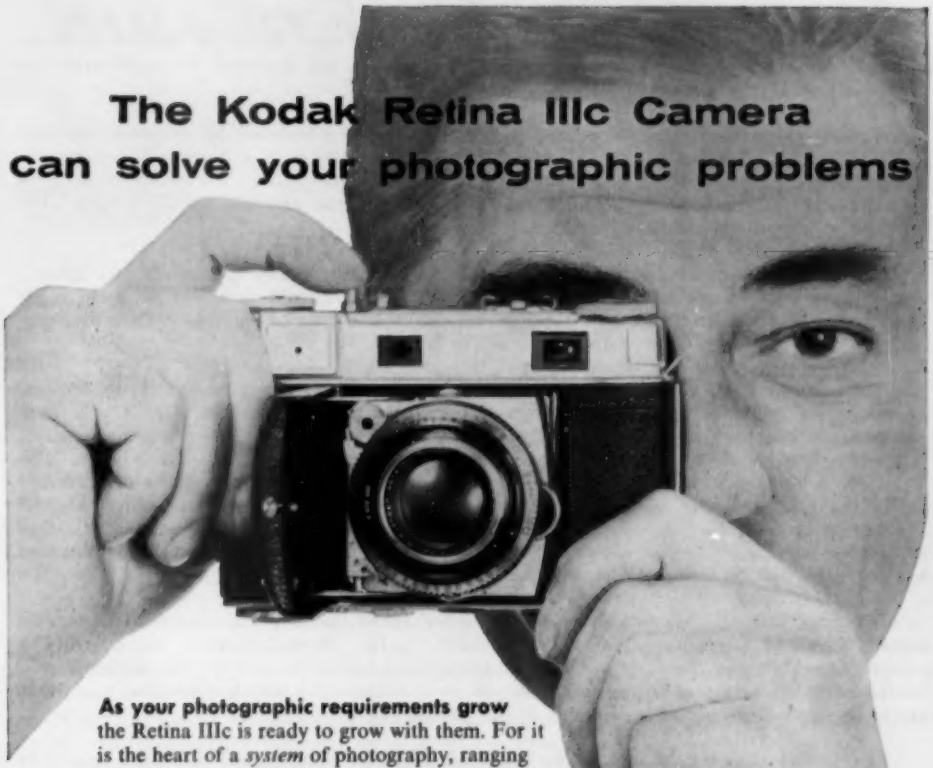
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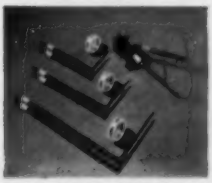
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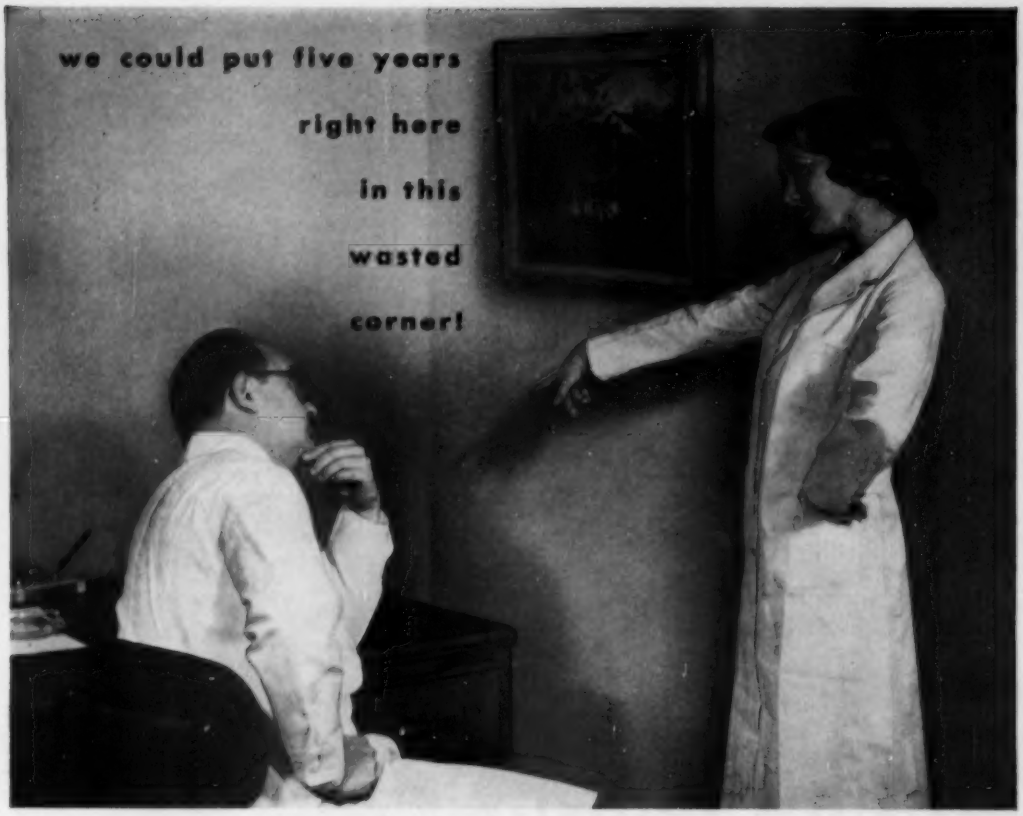
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Adrenal Lesions in Acute Meningococcemia

JOHN B. THOMISON, M.D., and JOHN L. SHAPIRO, M.D., Nashville, Tenn.

Though meningococcal infections are now generally well controlled by antibiotic therapy, cases of uncontrolled and rapidly fatal meningococcemia occasionally occur. These often exhibit the clinicopathological entity known as the Waterhouse-Friderichsen syndrome, which is by definition meningococcemia accompanied by purpura and peripheral vascular collapse with hemorrhage into the adrenal cortex.

As early as 1898 adrenal cortical hemorrhage was reported in a symposium of four papers appearing in the *Transactions of the Pathological Society of London*. Careful bacteriological studies had failed to reveal an etiologic agent, and one writer, Andrews,[1] stated:

> Assuming that it is an infectious disease, it must be by an organism which does not stain by the usual methods or grow on ordinary media. Variola at once suggests itself. Against this is the fact that there was no known source of infection, and no cases (of variola) subsequently arose in association with this.

In 1901 Little[2] collected 11 cases, including 4 of his own, and recognized as a clinical entity this condition of toxemia and purpura with the associated finding at autopsy of massive bilateral adrenal hemorrhage. Again, no etiologic agent was definitely identified, though he did demonstrate cocci, believed to be streptococci, in capillary thrombi in the skin lesions. Waterhouse,[3] in 1911, and Friderichsen, in 1918, reviewed the literature, and the latter gave an excellent pathologic correlation, but neither made any great contribution to the understanding of the disease. Although the Meningococcus had been isolated from a case of meningitis in 1887, it was not until 1916 that Maclagen and Cooke[4] reported the isolation of Meningococcus organisms in two cases of this more fulminating form of the disease.

The early reports were confined to cases of the typical "Waterhouse-Friderichsen syndrome." However, it became apparent that many patients died with purpura and vascular collapse due to meningococcemia, but at autopsy failed to show the anticipated adrenal lesions. In 1941 Rich[5] described a peculiar change, which he referred to as tubular degeneration, occurring in the adrenal cortex in severe septicemia, with meningococcemia as the classic example. Since that time this lesion has been reported with increasing frequency as a finding in acute meningococcemia.

Autopsies have been performed in 27 cases of acute meningococcemia at the Vanderbilt University Hospital. No series of comparable size from a single source appears in the literature, although a large number of cases in the files of the Armed Forces Institute of Pathology, drawn from many sources during World War II, and from a necessarily restricted age group, has been partially analyzed by Moritz and Zamcheck[6] and by Daniel.[7] We have had, therefore, a unique opportunity for the study of

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Read at a meeting of the Tennessee Society of Pathologists, April 9, 1956.

From the Department of Pathology, Vanderbilt University School of Medicine.



Fig. 1.—Gross photograph of adrenals, showing massive cortical hemorrhage.

the changes occurring in this disease. The present study is concerned only with the adrenal changes seen in fatal acute meningococcemia. Cases of chronic meningococcemia, with blood cultures persisting over a period of weeks or months, have not been considered.

Adrenal Lesions

The changes in the adrenal cortex in our cases were of three types: massive hemorrhage, patchy hemorrhagic necrosis, and tubular degeneration, alone or in combination.

1. *Massive Adrenal Hemorrhage.*—Bilateral adrenal hemorrhage is a striking finding when present, since the large, bluish retroperitoneal masses are quite obvious. The adrenals are much increased in size and show on microscopic examination the more or less complete ablation of the cortex by massive hemorrhagic necrosis. The hemorrhage affects first the zona reticularis, gradually migrating outward until the whole cortex is hemorrhagic. The medulla is characteristically spared. Four of our twenty-seven cases showed such findings (Figs. 1 and 2).

2. *Patchy Hemorrhagic Necrosis.*—Ten cases showed multiple small areas of hemorrhagic necrosis, in five instances associated with thrombosis of the sinusoids or larger vessels. Two of these showed damage which was estimated to be almost as great as that caused by massive hemorrhage. The three zones tended to be uniformly involved (Fig. 3).

3. *Tubular Degeneration.*—Most of the cases with patchy necrosis also showed extensive tubular degeneration, and nine other cases showed tubular degeneration alone. It has been postulated that tubular degeneration results from shrinkage of the cortical cells due to rapid depletion of the lipid hormone in response to body injuries. This shrinkage is thought to cause separation of the cell cords to form pseudotubules, which become filled with fluid exudate and cellular debris. In contrast to massive hemorrhage, this change begins in the zona glomerulosa and is more marked here, gradually moving inward (Figs. 4, 5, and 6).

The remaining four cases showed only minimal tubular degeneration and loss of

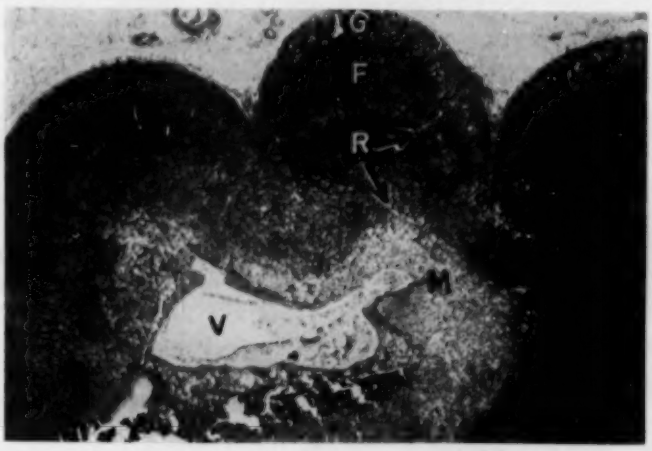


Fig. 2. — Massive adrenal hemorrhage. Though in this particular area stromal elements remain intact in the zona reticularis (*R*) and zona glomerulosa (*G*), no intact adrenal cortical cells can be made out anywhere in the cortex. The zona fasciculata (*F*) is completely destroyed. The medulla (*M*) shows no involvement. The irregular spaces at the bottom of the photograph are artifacts. *V* is the central vein of the adrenal. Hematoxylin-eosin stain; ×30.

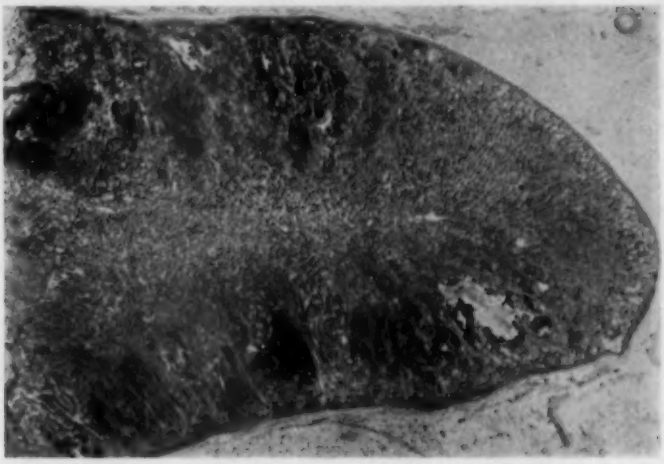


Fig. 3.—Multiple small areas of hemorrhagic necrosis in the adrenal cortex. Note also the tubular degeneration in the zona glomerulosa. Hematoxylin-eosin stain; ×15.

cortical lipids. In two of these cases the presence of significant lesions could not be definitely established.

Comment

In an attempt to relate severity of the adrenal lesions to the duration of the disease prior to death, we arbitrarily have designated as 1+ the milder degree of tubular degeneration, as 2+ the more marked tubular degeneration, and as 3+ the patchy hemorrhagic necrosis and the more marked tubular degeneration in conjunction with it. The designation 4+ we have restricted to massive bilateral hemorrhagic necrosis. The use of morphologic change as a yardstick of functional damage may be questioned, as may the assumption that the increment of damage is the same between 1+ and 2+, 2+ and 3+, and so on. Bearing in mind these limitations, one finds an apparent relationship between duration of the disease and severity of the adrenal lesion; the severer the lesion the more fulminating the disease.

The cases ranged from 4 to 220 hours in duration. All of the patients showing massive adrenal hemorrhage died within the first 24 hours, although two patients dying within 12 hours showed adrenal damage estimated at only 2+, as did the patient dying at 220 hours. Only one patient living more than 72 hours showed damage as great as 3+. The findings are summarized in Figure 7.

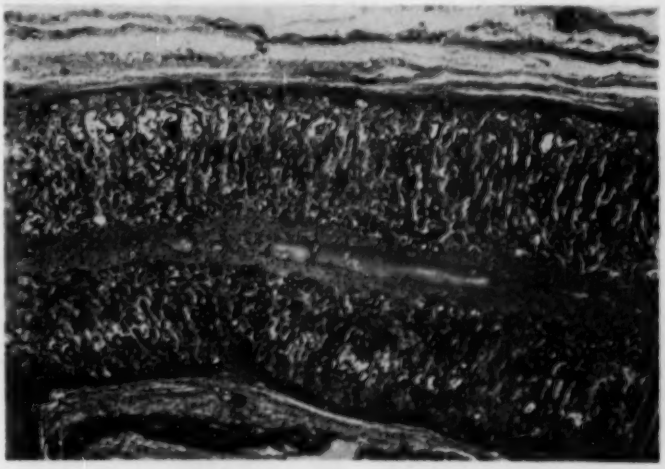


Fig. 4.—Severe tubular degeneration of the adrenal cortex. This is most marked in the upper portion of the zona fasciculata and zona glomerulosa, but involvement is widespread. The vessels of the zona reticularis are engorged, and the interstitial tissue shows a protein-rich edema fluid. Hematoxylin-eosin stain; ×40.

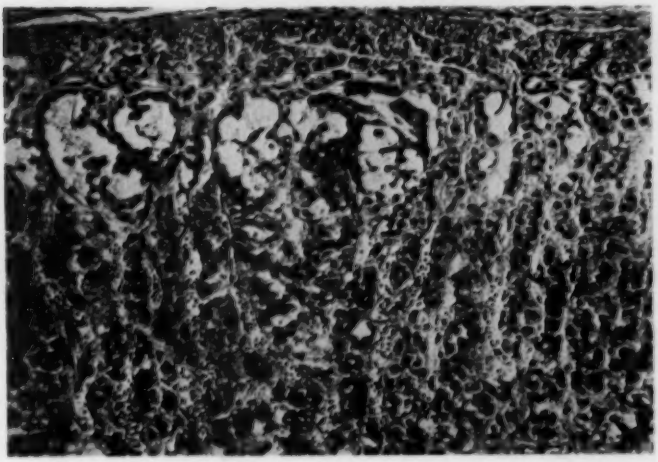


Fig. 5.—Tubular degeneration. In this particular area the severest involvement is in the upper zona fasciculata. Hematoxylin-eosin stain; × 150.

All of our cases of massive adrenal hemorrhage occurred in the first two and a half years of life, as is true of most reported series. In contrast, however, Moritz and Zamcheck,[6] in a series made up of 81 young soldiers, found 45 instances of bilateral massive adrenal hemorrhage. No other relationship is apparent between the age of the patient and either the severity of the adrenal lesion or the duration of the illness.

The majority of cases of acute meningococcemia occur in the very young. In this series 6 cases of fatal meningococcemia occurred in the first year of life, as against 15 cases distributed more or less evenly over the next 19 years. Only six cases occurred after the age of 20, the oldest patient being 54 years of age.

The mechanism of adrenal hemorrhage is not established by these studies. The findings seem to indicate that massive hemorrhage in the adrenal cortex occurs as a manifestation of severe, overwhelming infection by large numbers of organisms, with less severe hemorrhage in less severe infection. It is believed that the hemorrhage into the adrenals is due to the same mechanism as hemorrhage elsewhere: direct attack on the vascular endothelium by the organism or its endotoxin, or a combination of the two. It seems unlikely that adrenal hemorrhage is due to a loss of the support of the sinusoidal walls by the atrophied cell cords,

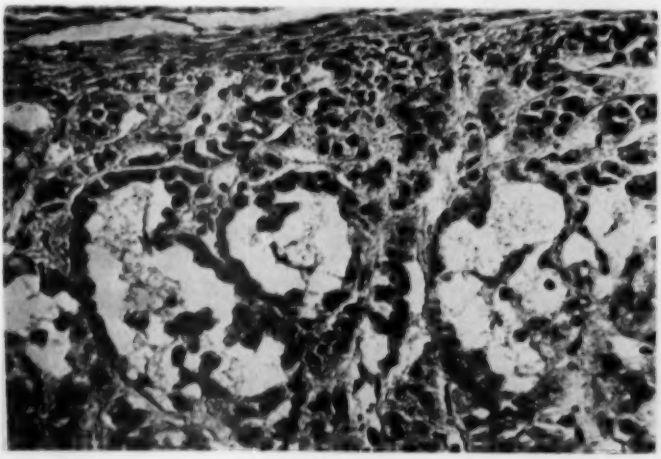


Fig. 6.—High-power photograph showing tubular degeneration. The cell cords are separated by exudate composed primarily of edema fluid, containing a few desquamated epithelial cells and occasional inflammatory cells. These tubules are primarily in the zona fasciculata, although there is a small one in the zona glomerulosa at the left border. Hematoxylin-eosin stain; × 300.

SEVERITY		TIME OF OBSERVATION			
		< 48hrs.	48–	≥72 hrs.	Total
<2+ = Minimal Lesions		8%	33%	62%	30%
2+or3+= Tubular Degeneration & or & Infarcts		46%	67%	38%	48%
>3+ = Massive Hemorrhagic Necrosis		46%	0%	0%	22%
Total	%	100%	100%	100%	100%
	Cases	13	6	8	27

Fig. 7.—Tabulation of data.

inasmuch as tubular degeneration begins in, and is severest in, the outer zones, whereas the inner zones are the site of the severest hemorrhage. It is not possible in the present study, with the material available, to evaluate the role which vitamin C and other factors known to affect the hemostatic mechanism might play in the hemorrhage in these cases.

Including the cases in the present series, 146 cases of proved meningococcemia have occurred in this hospital. Thirty cases clinically typical of the Waterhouse-Friderichsen syndrome have survived after therapy. In spite of the fulminating course, with peripheral vascular collapse and purpuric rash, recovery was complete within three to eight days, without residual effects. Although one frequently finds signs of transitory adrenal insufficiency, manifested by hypotension and relieved by the administration of adrenal cortical extract or steroid hormones, signs of prolonged adrenal insufficiency have not been encountered in those patients successfully treated in this hospital, nor is any such complication to be found in the literature. If massive adrenal hemorrhage had occurred in these patients, prolonged evidence of adrenal cortical insufficiency would be anticipated. It is of interest that two of our fatal cases showing massive adrenal hemorrhage were, at the time of death, receiving cortisone, as well as chemotherapeutic and antibiotic agents.

It seems warranted to conclude, therefore, that changes in the adrenal other than massive hemorrhage are reversible, with complete replacement of the destroyed glandular tissues and restoration of function. It is doubtful if patients ever survive infections severe enough to be accompanied by massive adrenal hemorrhage. The fact that death occurred in the face of adrenal replacement therapy and antibiotics emphasizes the overwhelming nature of the disease and the absolute necessity for early diagnosis and institution of treatment of the infection itself.

Summary and Conclusions

The lesions of the adrenal which occur in acute meningococcemia are presented. In 4 of the 27 cases reviewed there was no significant lesion.

It appears very likely that no patient survives bilateral massive hemorrhagic necrosis of the adrenal, which tends to occur early in the course of meningococcemia.

On the basis of these anatomical studies, as well as the clinical course of surviving patients, it seems that adrenal lesions other than massive hemorrhage are reversible, with complete restoration of adrenal cortical function.

Although acute meningococcemia may occur at any age, it is much commoner in young persons, particularly infants.

Mr. Ed Bridgforth, of the Department of Biostatistics, performed the statistical analysis on the data in this study.

Vanderbilt University School of Medicine (5).

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Cytochemical Effects of Azaserine on Ehrlich Ascites Tumor Cells

Action on Deoxyribose Nucleic Acid (DNA) and Total Protein

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An earlier report[4] on the influence of azaserine (O-diazoacetyl-L-serine) and 6-mercaptopurine on the metabolism of ascites tumor cells showed that azaserine inhibits the incorporation of glycine-2-C^{14} into acid-soluble and nucleic acid purines and enhances the utilization of adenine-8-C^{14}. Azaserine also caused a reduction in the mitotic index and gross cytological damage to the tumor cells during the time it was effective.

After six days of treatment with azaserine, these ascites tumor cells in Papanicolaou-stained smears showed rather large nuclei, which were suggestive of the possibility that this drug caused a blocking of mitosis while the DNA synthesis went on to produce higher-class cells (cells containing a higher amount of DNA in terms of Swift's DNA classes[19]).

We know this at present regarding the relation of DNA synthesis to mitosis: According to Swift,[19] in order to preserve DNA constancy, there appears to be doubling of DNA before there is any sign of mitotic activity. In the instance of diploid cells, 2C cells develop to 4C cells and then divide to become 2C cells again. "C" represents the amount of DNA in haploid spermatids. However, if normal mitosis is blocked by some mitotic poison, DNA synthesis might proceed to the formation of higher-class nuclei, such as octaploid or higher.

The purpose of this investigation is, therefore, that of determining whether the reduced mitotic index which was evident after azaserine treatment of the ascites tumor cells was due to interference with DNA synthesis or to mitotic blocking without a change in DNA synthesis. The latter would lead to polyploidy. With this in mind, the treated cells were studied for signs of abnormal mitosis and for relative amounts of DNA per cell.

In the earlier report[4] the azaserine-treated cells were noted as showing increased cell volumes. It seemed interesting to investigate whether this volume increase was due to an increase in total protein.

In the previous paper, no quantitative cytological findings were given. It is the purpose of this paper to present such findings.

Methods and Materials

Groups of Swiss mice were each injected intraperitoneally with 5×10^6 Ehrlich ascites tumor cells in saline suspension, as in the earlier experiment.[4] After 24 hours they were divided into two groups: a control group injected daily with saline, and a group injected daily with 20γ of azaserine per mouse. After six days of such treatment, peritoneal fluid was withdrawn from both treated and control mice. These mice were then killed, and the gross and microscopic autopsy findings were recorded, as previously described.[4]

After withdrawal of the ascites fluid, wet smears were prepared and fixed in methyl alcohol and Carnoy's fixative. All slides in this experiment were stained together, using the Feulgen technique,

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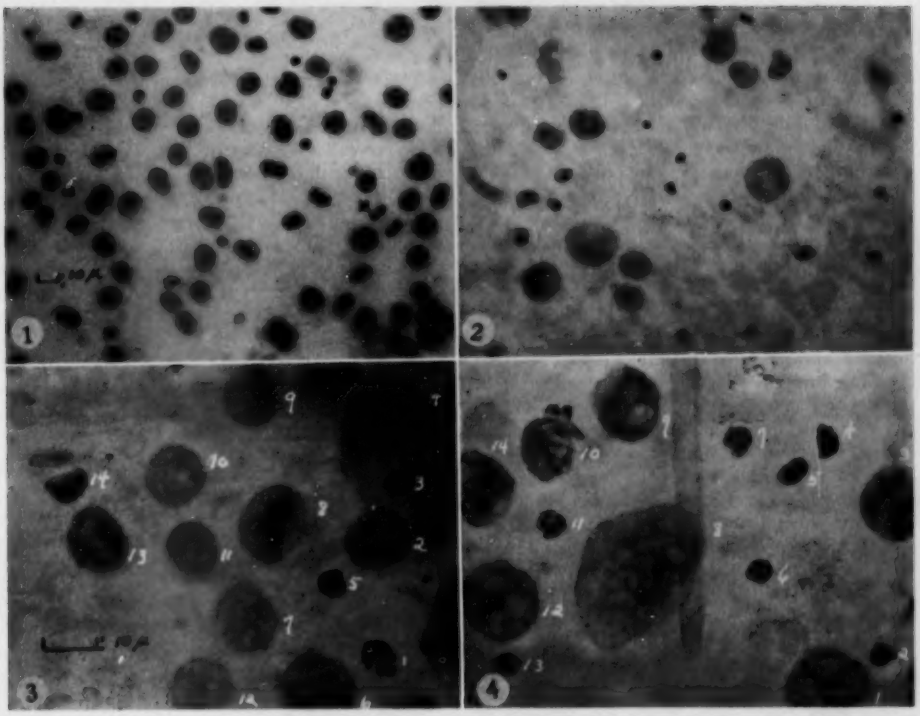


Fig. 1.—Ehrlich ascites tumor cells showing normal cell population with predominantly tetraploid cells, measuring 10μ-12μ in diameter. The Feulgen-stained nuclei were photographed with 550mμ wave length light. The density of the stain shows relative amount of DNA. Reduced to 80% of mag. $\times$ 350.

Fig. 2.—Ehrlich ascites tumor cells after six days of azaserine treatment, showing predominantly cells of 15μ-26μ diameter (swollen cells). The Feulgen-stained nuclei appear light as compared with those in Figure 1. The densities and the size of the photographed nuclei show an inverse relation between size and concentration of DNA after azaserine treatment. The few nuclei present show the reduction of tumor cells due to the effect of azaserine. The lymphocytes and leukocytes are increased in number, but show the same intensity of staining as in Figure 1. A large number of erythrocytes (hemorrhage) is present. Reduced to 80% of mag. $\times$ 350.

Fig. 3.—Feulgen-stained ascites tumor cells as in Figure 1 shown at a magnification of $\times$ 1000. Nuclear membranes and the fine network of chromatin granules and filaments are well demonstrated. Cells numbered *2, 9, 10, 11,* and *13* are tetraploid cells with relative amounts of DNA of about 20 (arbitrary units). Cells numbered *3* and *5* are lymphocytes and have a DNA value of about 10; mesothelial cells, numbered *1* and *14,* show the same value (diploid cells). Cells numbered *6* and *8* are octaploid cells, giving a DNA value of about 39. Cells numbered *7* and *12* are intermediate cells, having a DNA value of 27 and 30. Reduced to 80% of mag. $\times$ 1000.

Fig. 4.—Feulgen-stained azaserine-treated ascites tumor cells as in Figure *2* but at a magnification of $\times$ 1000; reduced to 80% of magnification. Nuclear membranes, chromatin granules, and filaments are prominent and form bands around large intranuclear vacuoles. Cells numbered *2, 6,* and *13* are lymphocytes, and cells numbered *4, 5, 7,* and *11* are leukocytes—all giving the same diploid DNA value (arbitrary units) of about 10; this is the same as the lymphocytes in Figure 3. Cells numbered *1, 9, 10,* and *14* give a relative DNA value of 22 to 24 (swollen tetraploid cells). Cells numbered *3* and *12* give DNA values of 36 and 39 (octaploid values). Cell numbered *8* gives a DNA value of 56 (degenerating high octaploid).

with 12 minutes' hydrolysis, and the cold Schiff procedure according to Lillie.[10a]

For comparison of these ascites tumor cells with a known standard, slides of beef liver cells were included for Feulgen staining, as has been described by Leuchtenberger.[13] As a means of checking the specificity of the Feulgen reaction, one slide was processed without hydrolysis.[10] For demonstration of basic groups of protein, naphthol yellow S stain was used at pH 2.78.*

The following methods were used to demonstrate the cytological picture seen on the stained smears:

* Miss Evelyn Welch and Miss Sally Smith gave technical assistance.

1. The stained slides of this and of the previous experiment were examined under the light microscope and differential counts and nuclear-size measurements done to obtain a picture of the prevailing cell population in both the control and the treated mice. The differential counts were made with the special purpose of measuring the diameters of the nuclei, in microns, to aid in assigning them to different DNA classes.

2. Qualitative histochemical examinations were done on Feulgen-stained slides of this and of the previous experiment to get an idea of the DNA content. This was done by a survey with the light microscope at 550mμ wave length illumination, which is required when using Feulgen-stain intensity as a quantitative measure of the DNA content of nuclei. Feulgen-stained nuclei of the treated and untreated ascites cells were selected at random and evaluated for differences of density, especially between the large treated cells and the untreated cells (Figs. 1 and 2). For total protein the naphthol yellow S-stained slides were examined at 450mμ wave length illumination.[9]

3. Quantitative microspectrophotometric measurements were done on a random selection of nuclei to confirm the observations from the above methods. To measure the intensity of the Feulgen stain as a quantitative measure of DNA, the nuclei were studied with a microspectrophotometer.[34,35]

For the microspectrophotometric measurements, 550mμ wave length light, which was obtained by using a tungsten-ribbon filament lamp in conjunction with Kodak Wratten Filters No. 21 and No. 61, was used to get the maximum light absorption of DNA. Relative amounts of DNA in the nuclei were calculated by multiplying the extinction by the nuclear area.[11,19]

4. For more detailed observation of the nuclei and for a permanent record of this, photographic densitometry[8,7,16,†] was employed, using a transmission densitometer calibrated in terms of ASA transmission diffuse density (ASA Standard Z38.2.5-1946). For the photomicroscopy, Kodak Super Panchro-Press Type B Film (3¼×4¼ in.) was used.[9] Illumination of the Feulgen slides was provided by 550mμ wave length light. The lamp and camera were aligned on the optical axis of the microscope for uniform illumination of the field. Exposure times were chosen to given density measurements on the straight-line portion of the characteristic curve of this film. Areas selected at random from each slide were photographed.

Processing of the exposed film was carried out according to the procedure recommended by Kodak Films[9] under carefully controlled conditions of temperature, constitution of the developer, agitation, and time of development. Groups of negatives were processed together.[35]

The density measurements with the transmission densitometer were determined by comparing the density of each nucleus with the density of the background of the negative. The log scale of the densitometer thus showed the density in ASA diffuse density units. For purposes of measuring the nuclear diameters, a stage micrometer was photographed at the same magnification (98×12.5) as the cells. For computing the relative amount of absorbing substance (DNA) in each nucleus, the formula E (πr^2) was used.[11,19] The diameters were taken directly from the negatives and the extinctions computed by taking the mean of the extinctions of several 2 mm. plugs through each nucleus. All nuclei seen on each negative were so evaluated.

Results

1. Differential counts were made of a random sample of control ascites tumor cells using an ocular micrometer, calibrated on a stage micrometer, for measurements of nuclear diameters. This showed the prevalence of 10μ-12μ nuclei (about 82%), with a small number of 15μ nuclei (about 13%). No 20μ nuclei were seen; about 5% of cells in mitosis were observed. Mesothelial cells, histiocytes, lymphocytes, and leukocytes were not included in this differential count.

A similar count made of azaserine-treated tumor cells showed only a small group (about 11%) of 10μ-12μ nuclei, while larger nuclei had increased, those of 15μ diameter making up about 58% and of 20μ about 24%. A few (about 6%) even larger cells containing large vacuoles were noted. Mitosis was decreased to about 1%. No abnormal mitoses were seen.

2. A qualitative histochemical evaluation of these Feulgen-stained nuclei was done at the time of determining the above measurements, using 550mμ wave length light to demonstrate the intensity of the nuclear stain. In the control ascites tumor cells, the intensity of the stain appeared to be the same in the two sizes of nuclei, those of 10μ-12μ diameter apparently belonging to the tetraploid class and those of 15μ to the octaploid class. The over-all intensity

† Drs. A. E. Whitford and A. D. Code, of the Department of Astronomy, collaborated in the photographic technique, and Mr. Charles Mohr assisted in the photography.

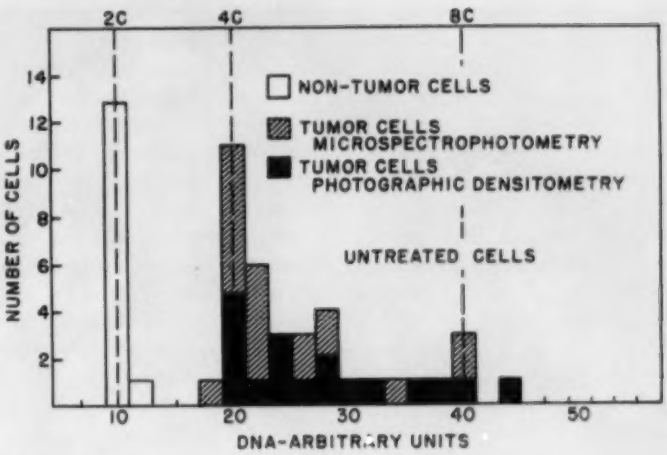


Chart 1.—Untreated Ehrlich ascites tumor cells six days after transplantation, shown in relation to DNA classes. DNA measurements of interphase cells. Black and shaded areas, tumor cells; unshaded areas, nontumor cells (lymphocytes, leukocytes, mesothelial cells, and beef liver cells), as reference for diploid DNA value (2C).

of the stain of this control group of nuclei made them appear about twice as dark as those from mice treated with azaserine (Figs. 1 and 2). In scanning numbers of azaserine-treated tumor cells at random, it was apparent that the intensity of the stain decreased with the increase in cell size.

With the naphthol yellow S stain for protein the findings were different. The enlarged, azaserine-treated cells stained almost as densely yellow as the small, control cells, indicating an increase in the protein content per cell.

3. Quantitative microspectrophotometric measurements were made first on a random sampling of Feulgen-stained ascites tumor cell nuclei from the control group with the aim of assigning them to Swift's DNA classes using beef liver nuclei, as well as mouse diploid cells, represented by the lymphocytes, leukocytes, and mesothelial cells found on each slide. The DNA content

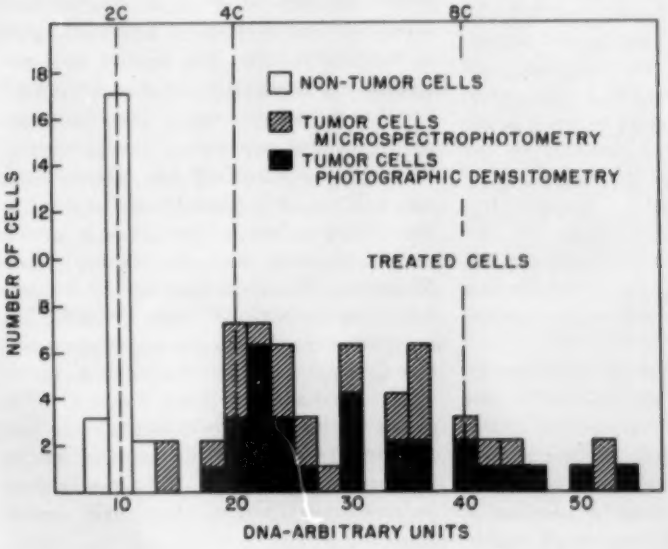


Chart 2.—Azaserine-treated Ehrlich ascites tumor cells six days after transplantation. The frequency distribution shows that the same DNA classes (tetraploid, intermediate, and octaploid cells) predominate similarly to the untreated cell population. Black and shaded areas, tumor cells; unshaded areas, nontumor cells.

per nucleus of the control tumor cells appeared approximately twice that found in normal beef liver nuclei, such as was found by Leuchtenberger,[12] and, therefore, established them as tetraploid cells. The same relationship between these tetraploid cells and the mouse diploid cells was observed. The diameters of most of these cells measure 10μ-12μ. Distributions of cells of both control and treated groups as to DNA classes, and as compared with beef liver cells and lymphocytes and leukocytes, are shown in Charts 1 and 2. In addition to tetraploid cells, octaploid and intermediate cells were found; the frequency distribution of these ascites tumor cells was similar to that described by Leuchtenberger [12] and Richards.[16]

After this, a group of nuclei from the treated ascites tumor cells were measured. When the measurements were compared with those of the control group, these nuclei showed (Chart 2) no increase in relative amounts of DNA in spite of increase in cell diameters, the mean for the determined relative DNA content for 4C cells being almost identical in the two groups, whereas the nuclear diameters ranged from 10μ to 12μ, in the control group, to 15μ to 25μ, in the treated group. This would seem to indicate that the nuclei with larger diameters represent only enlarged, and possibly degenerating, nuclei of the normal cell population. The azaserine apparently does not produce cells of a higher DNA class, as in such a case we should expect to find a larger number of nuclei with a doubling of the DNA content to 8C and 16C values.

4. With photographic densitometry, measurements of the densities of the photographic negatives of the Feulgen slides showed about the same class distribution as that found with the microspectrophotometric method.

In addition, the morphologic structures of the nuclei were well demonstrated. All nuclear membranes were prominent. In the untreated, interphase cells (Fig. 3) the chromatin granules and filaments were distinct and evenly distributed, forming a fine network which left very small spaces for the lightly stained nuclear sap. The picture was quite different in the enlarged azaserin-treated nuclei, where the chromatin granules (Fig. 4) were even more prominent but more widely separated. It appeared that the chromatin granules and filaments were pushed aside by the formation of large lightly stained, nuclear sap-filled vacuoles. However, the number of chromatin granules appeared to be about the same in the untreated and in the treated cells. As only the chromatin granules and strands of the nuclei stain deeply Feulgen-positive, they represent all the DNA content of the nuclei.

The total amount of DNA as represented by these chromatin granules and strands in the treated cells does not appear increased as compared with that in the untreated cells. The increase in volume in these treated cells is apparently due to the increase in nuclear sap, which according to Brown [1] consists of a complex basic protein and is devoid of nucleic acid.

Photographic densitometry on randomly selected cells of the naphthol yellow S-stained slides was carried out in the same way as for the Feulgen-stained slides. The naphthol yellow S dye concentration was rather uniform in most of the untreated cells; however, it appeared somewhat lighter in the treated cells. The relative total protein values appeared to be directly proportional to the cell diameters. The relationship of the relative amounts of total protein of the untreated tetraploid and octaploid cells was 1:2, similar to that found for the relative DNA values. These values are in agreement with findings obtained from Millon-stained cells as described by Schrader and Leuchtenberger [17] and Swift.[20] The azaserine-treated cells showed higher relative protein values, corresponding to the larger cell diameters (Figs. 5 and 6). The group of the markedly enlarged cells (cell diameters from 20μ to 30μ) showed relative total values up to five or six times as great as was found for the tetraploid, control cells.

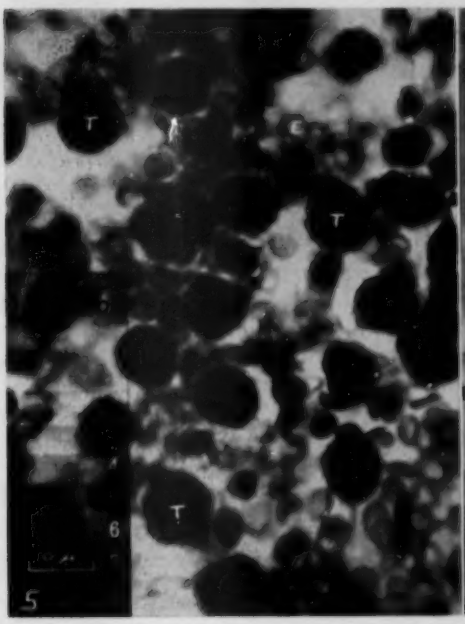


Fig. 5.—Ehrlich ascites tumor cells of saline-treated (control) mouse stained for total protein with naphthol yellow S and photographed with 450mμ wave length light. The densities of the tumor cells (*T*) show the relative amounts of total protein per cell. Many faintly stained erythrocytes (*E*) are seen. Reduced to 94% of mag. × 1000.

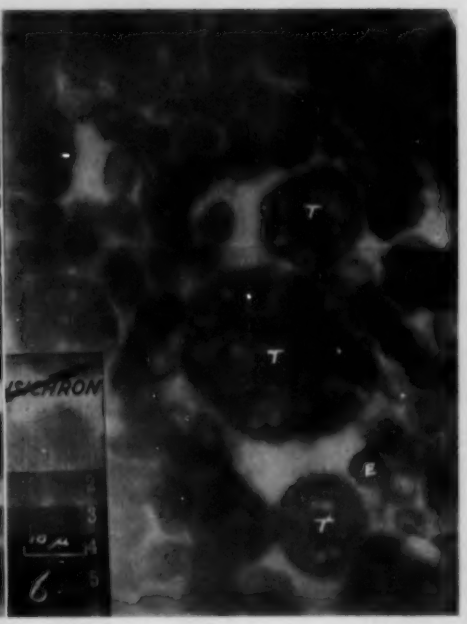


Fig. 6.—Ehrlich ascites tumor cells (*T*) after six days of azaserine treatment. These cells were stained and photographed together with the cells shown in Figure 5. The large cell in the center has a diameter about three times as large as that seen in many of the untreated cells. The large cells, again, show small vacuoles and stain about one-third lighter than the control cells. Reduced to 94% of mag. × 1000.

5. The mice selected for the total cell count experiments with azaserine were Swiss mice and weighed from 25 to 30 gm. After injections with 5×10^6 tumor cells in saline suspension, the control mice usually increased their weight by 4 to 8 gm. The volume of the ascitic fluid obtained from these control mice treated daily with saline injections varied from 2 to 4 cc. One mouse out of three usually failed to gain weight and to produce a comparable volume of ascitic fluid or to show the usual rise in tumor cell count[8] from 5×10^6 to 5×10^8 within the seven days of the experiment. This is assumed to be due to some leakage of fluid after intraperitoneal ascites tumor cell injection. The azaserine-injected mice usually gained less weight, not more than 3 gm., and the amount of ascitic fluid averaged 2.3 cc. After six days of 20γ of azaserine/mouse/day, the total cell count was reduced in a selected case from 394×10^6, in the control mouse, to 73×10^6, in the treated mouse. This would represent a reduction of cells in a 4:1 ratio. It is interesting to point out that the mitotic index dropped from 4.1 to 1.5 during the same treatment.[4] In this series of experiments, survival time was not studied, as the animals had to be killed in order to determine the total volume of the ascitic fluid.

Comment

The morphological, and qualitative and quantitative histochemical examinations of azaserine-treated ascites tumor cells would seem to indicate that the action of azaserine involves inhibition of DNA synthesis rather than blocking mitosis. In the larger cells appearing in the azaserine-treated mice the DNA concentration per cell nucleus is de-

creased proportionately as the volume increases. The relative amounts of DNA in treated and in untreated cells are about the same, showing the independence of the DNA of nuclear size and nuclear volume. This relation was called constancy of DNA by Pollister.[15]

The finding of DNA classes with tetraploid cells, octaploid cells, and cells with amounts of DNA ranging between these two values (intermediate cells) is consistent with Swift's theory of DNA doubling during interphase.[19]

The cytochemical findings in this study are in agreement with the studies of nucleic acid synthesis in ascites tumor cells, as done by LePage and associates.[16]

The protein findings showed a different pattern. After azaserine treatment the enlarged cells appeared to have an increased amount of protein per cell, indicating no inhibition of protein synthesis. Generally the total protein values were found to be directly proportional to the cell volume. It is interesting to note that Swift[20] found much the same pattern of enlarged nuclei with DNA-synthesis inhibition and continuing protein synthesis in his experiments with mechlorethamine hydrochloride, sterile N. F. (nitrogen mustard) on onion-root tips.

The morphological changes resulting from treatment of Ehrlich tumor cells with azaserine are like those reported by Zollinger,[22] in describing early stages of dying cells. The morphological azaserine findings are also similar to those described by Gusberg[5] as irradiation effects on human cervix cancer cells, with increased nuclear size and vacuolation prominent. Massive steroid therapy of leukemia has produced similar cytological changes, as recently reported by Hill.[6] The azaserine effect should, therefore, be considered as nonspecific and probably indicating only early degeneration of cells.

Summary

When Ehrlich ascites tumor cells are treated with azaserine (20γ per mouse for six days), there is a reduction in the mitotic index (from approximately 4.1 to 1.5), paralleled by a marked decrease in total tumor cell count. The remaining tumor cells are enlarged but show a decreased concentration of deoxyribose nucleic acid (DNA). The total protein content of these cells is increased. These changes are probably due to DNA synthesis inhibition.

These findings are obtained by comparing ascites tumor cells from azaserine-treated mice with cells from control animals injected with saline. Smears of these cells are stained by the Feulgen method and examined with 550mμ wave length light to evaluate the DNA content per cell. For total protein determinations naphthol yellow S-stained cells are examined with 450mμ wave length light. Relative amounts of DNA and total protein are determined using microspectrophotometric and photographic densitometric methods.

Morphological changes observed appear to resemble changes associated with early cell death and would seem to indicate that azaserine probably has a nonspecific effect on Ehrlich ascites tumor cells.

Dr. G. A. LePage, professor of oncology, McArdle Memorial Laboratory, and Dr. W. D. Stovall, director, State Laboratory of Hygiene, gave advice and help in this study.

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Pathology of Radioisotopic Ablation of the Thyroid in the Dog

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There has been a difference of opinion in recent years concerning the metabolic role of the thyroid gland in the dog. According to a recent report,[1] myxedema can be produced in the radiothyroidectomized dog, even though previous investigators have not consistently observed the usual manifestations of hypothyroidism in dogs following thyroidectomy or thiouracil administration.[2-5] In conjunction with other studies of the thyroid concerning protein metabolism, the effects of the internal ionizing radiation of I^{131} have been investigated in mongrel dogs to determine (1) whether the thyroid in this species has an important role in the animal's metabolism, (2) the pathology of canine myxedema, and (3) whether selective localization of this radioisotope might in time result in the production of a neoplasm in the thyroid. The last-mentioned possibility is a problem of importance in considerations of the justification of the clinical usage of radioisotopes for diagnostic purposes in man. (Reported studies of possible experimental carcinogenesis from I^{131} in the past have been largely confined to laboratory rodents.)

Materials and Methods

Eight adult mongrel dogs (five male, three female) were studied. They were maintained principally on a ration of commercial, dried dog food, which iodine analysis showed to contain 0.14γ of iodine per gram of foodstuff. Pork lard was added, making the proportion of fat about 30% of the total calories. At the beginning of the study the animals were given a tracer dose of 99μc of carrier-free I^{131} intraperitoneally, and complete fecal and urinary collections were made in metabolism cages at daily intervals for four days. Urine and feces were counted in a well-type gamma scintillation counter against the same volume of a suitable dilution of the administered radioiodine solution. Radioactivity was calculated as the fraction of the administered dose. At the end of four days the total retention of isotope ranged from 0.27 to 0.67 of the administered dose, with a mean of 0.43. The average values of the rate constants of renal excretion (K_{1e}) and of thyroid uptake (K_{1t}) calculated by the approach outlined by Keating[6] were 0.49 and 0.31 per day, respectively. The average value of the four-day fecal excretion was 0.026.

To assure maximal thyroidal uptake of the ablative dose administered, two weeks after the tracer all animals were given only distilled water for a period of four days preceding and three days subsequent to administration of the ablative dose. The fast was expected to accomplish two things: (1) increase thyroidal uptake of I^{131} by decreasing iodine intake and depleting body stores of iodide, and (2) markedly reduce the urinary excretion of administered I^{131} ion and thereby increase thyroidal uptake. The second effect was expected from the studies of Riggs,[7] who noted a marked dependence of iodide clearance in the dog on chloride excretion, with prolonged retention of iodide by dogs whose kidneys were conserving salt.

On the fourth day of the fast the animals were given I^{131} admixed in about 50 gm. of ground horse meat. Ingestion appeared to be virtually quantitative. Three days later the regular diet was resumed. Following administration of the isotope, urine was collected at 1-3 day intervals for 19 days. A single accumulated 10-day fecal collection was made. Methods of collection of excreta and quantitation of radioactivity were the same as those described above for the tracer study. The data on animal weights and magnitude of the doses are given in Table 1. The complete clinical and biochemical studies on these animals will be reported later in a separate publication, so that techniques in relation to those investigations are

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TABLE 1.—*Animal Weights and Doses of I^{131}*

Dog No.	Body Weight, Kg.	Estimated Thyroid Weight, Gm.	Millicuries Administered	Millicuries per Kilogram Body Weight	Administered Microcuries/Gram Estimated Thyroid Weight
A-58	23.2	1.43	2.20	0.094	1,520
A-61	18.8	1.19	1.65	0.088	1,390
A-63	26.2	1.63	1.65	0.063	1,010
A-60	36.0	1.61	16.5	0.63	10,200
A-62	23.7	1.48	13.8	0.58	9,300
A-66	31.2	1.84	22.0	0.71	12,000
A-68	28.7	1.77	19.3	0.67	10,900
A-64	22.4	1.40	22.0	0.98	15,700

not recorded here. After 14½ months of observation the animals were killed, and complete autopsies were performed. Particular attention was paid to making block dissections along the course of the larynx and trachea to locate any possible residual thyroid tissue (both for counting sections and for autoradiographs). Only routine stains (hematoxylin and eosin; the Van Gieson, Weigert, and periodic acid-Schiff methods; Rinehart's acid mucopolysaccharide; Best's carmine, Mallory's phosphotungstic acid-hematoxylin, and Mayer's mucicarmine) were employed for the tissues studied microscopically.

Results and Analysis of Data

Radiation Dosage to the Thyroid.—The marked efficacy of fasting in reducing excretion of iodide was indicated by the fact that the cumulative I^{131} excretion in the three days following administration of the ablative doses was less than 0.05 of the dose in six dogs and amounted to only 0.09 and 0.12 in the other two. Excretion of isotope was accelerated after resumption of the diet on the third day, but was considerably more rapid in the high-dose than in the low-dose animals. These findings are shown graphically in Figure 1.

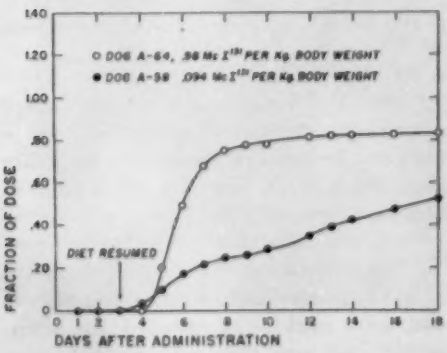


Fig. 1.—Cumulative urinary excretion of thyroid ablation doses of I^{131}.

It was desirable to estimate, if possible, the radiation doses to the glands. Since in vivo counts over the glands during treatment were not feasible, the excretion curves were the only available data from which the time course of isotope in the glands could be estimated. It was felt that the estimates obtained by the following approach, though very crude, were of value in indicating the order of magnitude of the actual radiation doses received by the glands.

The studies by Feller et al.[8] of I^{131} ablation of the rat thyroid showed no reduction in peak uptake in glands of animals concentrating as much as 16,000μc per gram of gland, compared with animals receiving tracer doses. Virtually complete loss of further iodide-concentrating capacity, however, was demonstrated by tracer doses given the treated animals on the third day. Net loss of isotope from glands with peak uptakes of about 5600μc per gram began at about 48 hours, and from glands with peak uptake of about 16,000μc per gram at 24 to 48 hours. The rate of loss was greater in the high-dose animals.

The data of Rall and Berman[9] relative to in vivo counts over the thyroid glands of six patients given thyroidectomizing doses of I^{131} were examined. Peak uptakes ranged from 90μc to 1500μc per gram of estimated thyroid weight. In all cases the decline of isotope in the glands could be fitted to a single exponential, with the fractional rate of loss ranging between 0.020 and 0.79 per day.

The data of Robbins et al.[10] for man and that of Tong et al.[11] for the rat suggest that

TABLE 2.—*Range of Dosage and Maximal Gland Uptake of I^{131}*

Dog No.	Exponential Constants from Urinary Excretion Curves (per Day)			Estimated 72-Hour Uptake Fraction of Dose	Estimated Range of Total Beta Radiation Dosage, rep/10^3
	a_1	a_2	a_3		
A-58	0.86	0.40	0.094	0.97	31–102
A-61	0.90	0.47	0.23	0.87	33–55
A-63	0.86	0.48	0.10	0.95	26–46
A-60	----	0.54	0.29	0.97	280–441
A-62	----	0.64	0.28	0.99	310–420
A-66	1.58	1.28	0.27	0.97	310–530
A-68	1.69	1.22	0.25	0.99	300–520
A-64	1.36	0.87	0.38	0.99	440–630

the principal labeled organic compounds of iodine appearing in the plasma after thyroid-destructive doses of I^{131} are thyroxine and thyroglobulin.

The rates of loss of these compounds from the animal due to catabolism and excretion are not equal. From the published data of tracer studies by Brown and Jackson,[12] Gross and Leblond,[13] and Albert,[14] one may compute the fractional rates of loss of thyroglobulin, thyroxine, and iodide as about 0.059, 0.034, and 0.050 per hour, respectively, in the rat. In the labeled thyroxine studies, about 50% of the total excretion of the I^{131} label was by the fecal route.

With these considerations in mind, a three-compartment kinetic system was assumed. The partial solution of this system, detailed in the Appendix, afforded an estimate, based on analysis of urinary excretion curves, of the possible range of the dosage to the glands, in reps, and an estimate of maximal gland uptake of the isotope. The results are given in Table 2.



Plate I

Dog before and 14 months after administration of I^{131}.

Clinical Response

After administration of the ablative dose of I^{131} the animals were observed for 14½ months. During this period each of the dogs developed well-marked myxedema. Clinically, the alterations in hair and skin were striking and began to be noticeable within two to three months. Epilation usually began in the periorbital region, and the final total area of skin devoid of hair varied from about 50% to 98%. Widespread desquamation became prominent, and the skin felt thickened. The appearance of one of these dogs 4 weeks and 14 months after receiving the radioisotope is presented in Plate I. This animal shows the marked alterations (elephantoid appearance) in the skin and the lethargic appearance, not unlike that observed in human myxedema. Cutaneous infection with ulceration occurred in some dogs. Conjunctivitis was also noted.

With progression of the disease there was a spontaneous reduction in bodily activity and food intake. In spite of the latter, within the first three months the mean increase in body weight was 18%. Hyper-

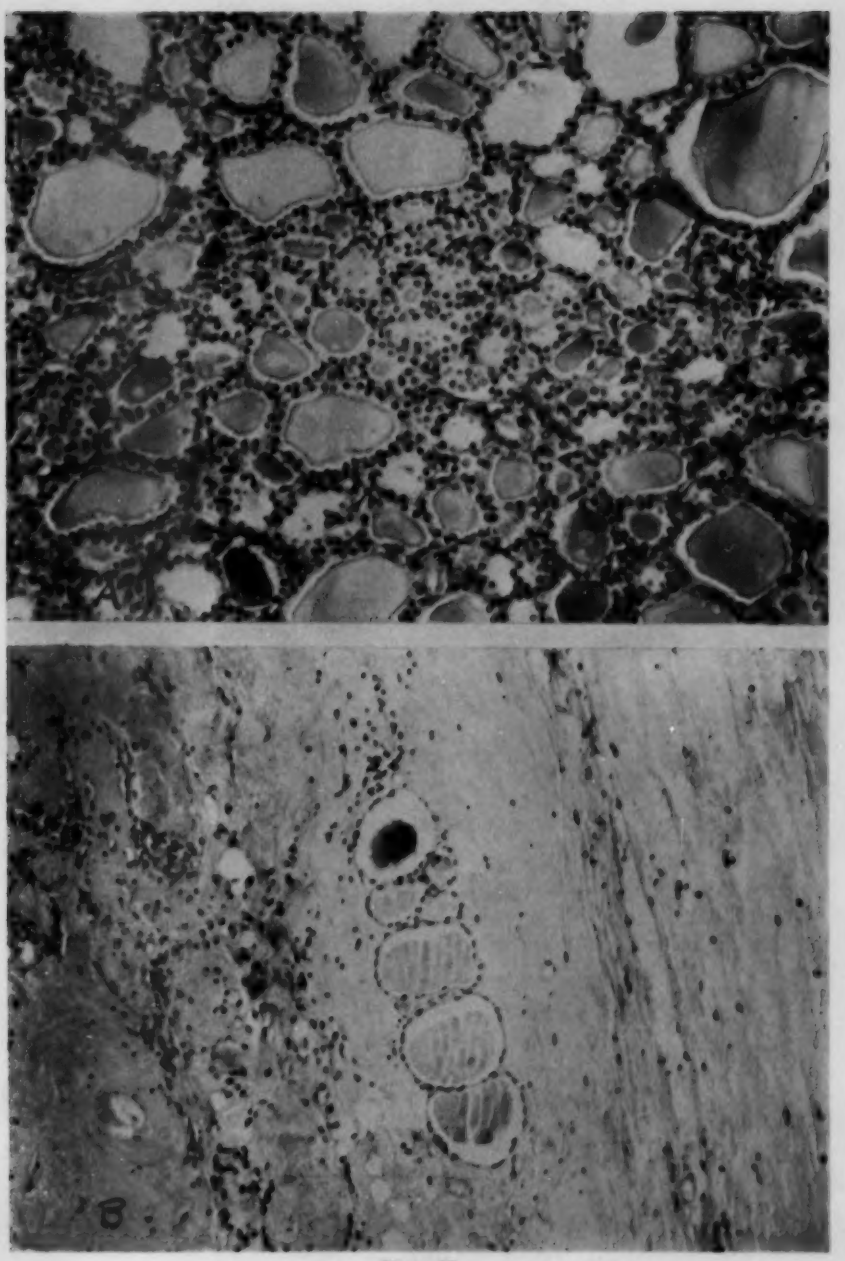


Plate II

A, normal thyroid in adult dog. *B*, thyroid in myxedematous dog. Hematoxylin and eosin stain; × 80.

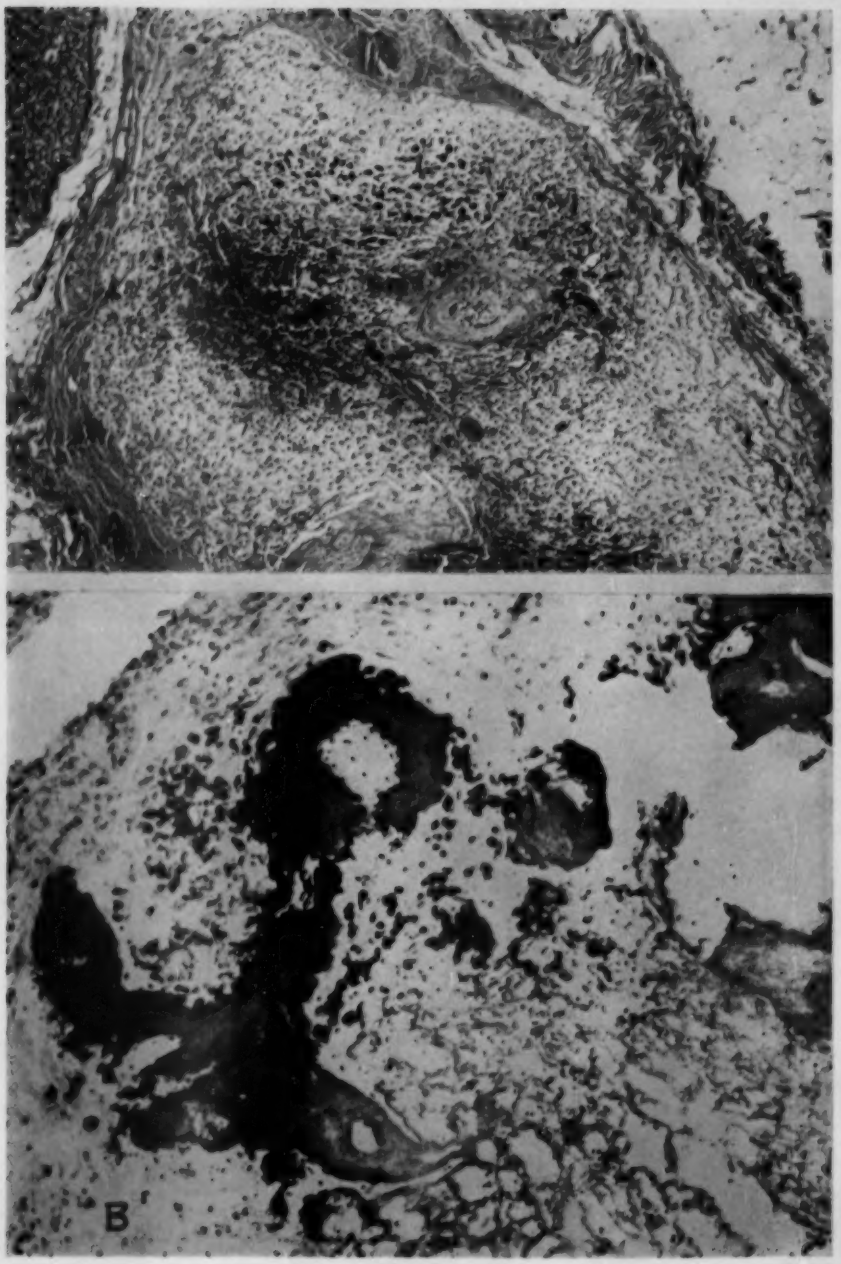


Plate III

A and *B*, thyroid in myxedematous dog. Hematoxylin-eosin stain; × 80.

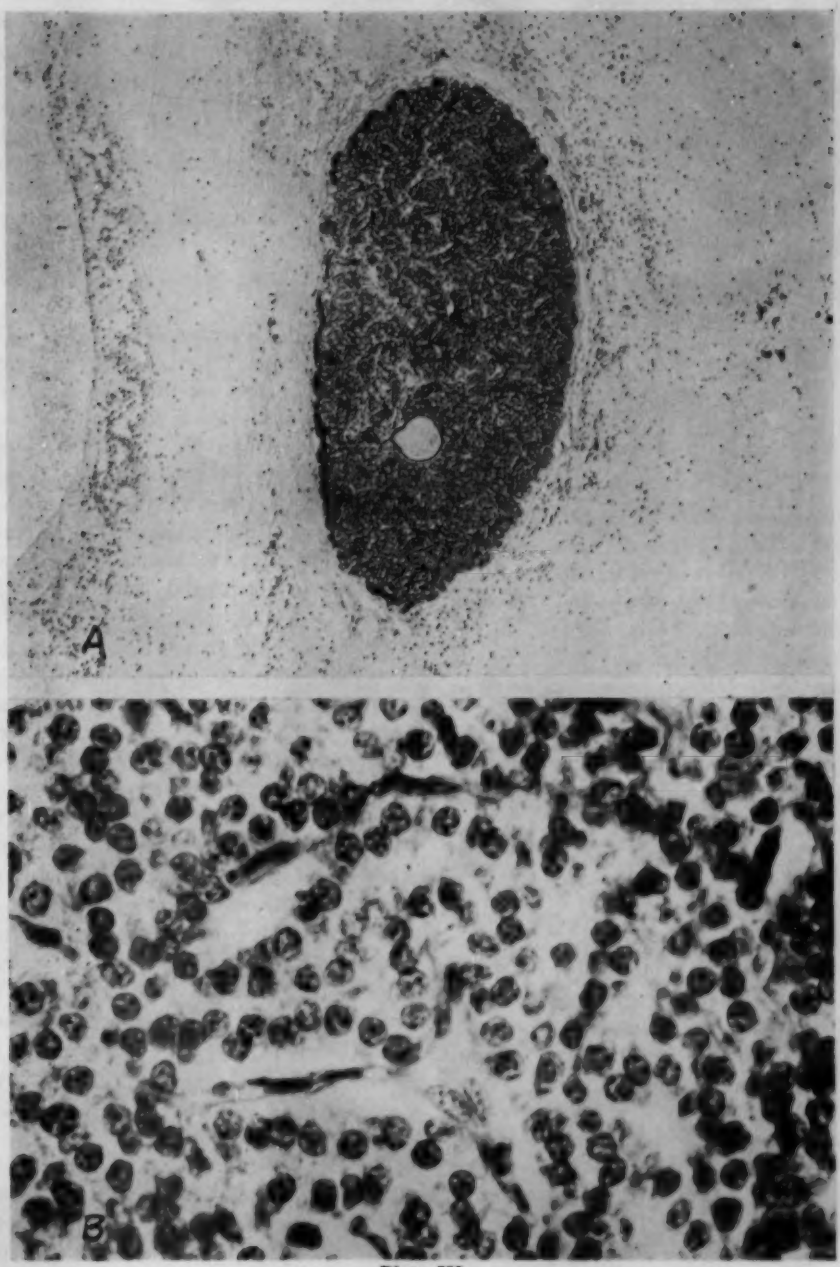


Plate IV

A, intact parathyroid in radiothyroidectomized dog; × 80. *B*, parathyroid from same animal as *A*; × 320.

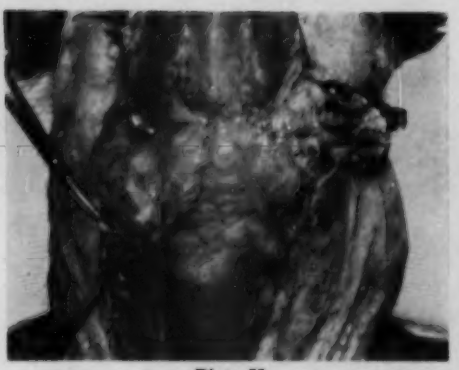


Plate V

Gross appearance of carcinoma of thyroid in radiothyroidectomized dog.

cholesteremia and hyperglobulinemia developed in all animals.

Results of Pre-Killing I^{131} Tracers

In the five animals in which I^{131} thyroid uptake was quantitated by scintillation counting of the resected tissue, the uptake in all was found to be negligible. The highest value obtained was 0.00014 of the administered dose. Autoradiographs of thyroid-area tissue in six animals showed no detectable radioactivity.

Pathology

The most important pathologic finding in all of the dogs examined was the effect of I^{131} upon the thyroid. Owing to the fact that block dissections were made through the entire region where thyroid tissue might normally exist, it was possible to locate microscopic remnants of thyroid in each animal. The largest single recognizable group of follicles did not cover an area greater than three low-power microscopic fields. In this case some of the follicles appeared essentially intact, while the majority were in various stages of degeneration. The degenerative features of thyroid ablation by I^{131} are demonstrated in the following series of plates. For comparative purposes, the microscopic appearance of the thyroid from a control mongrel dog is shown in Plate II*A*. Plate II*B* demonstrates the paucity of follicles, found only after a careful search in each dog. Such scattered follicles are small, lined by low cuboidal epithelium, and encompassed by dense collagen. Autoradiographs of adjacent sections disclosed no uptake of I^{131}. Further evidences of the marked degenerative changes that occurred in the thyroid are seen in Plate III*A* and *B*. In the former, pigmentation, fibrosis, and vascular hyalinization are prominent, and in the latter, calcification. The parathyroid gland is intact (Plate IV*A* and *B*).

In one dog, clinically and at autopsy, a large, firm mass was noted in the region of the thyroid, as shown in Plate V. The neoplasm had ulcerated through the overlying skin surface. Microscopic examination of the lesion showed it to be an adenocarcinoma of the thyroid (Plate VI*A* and *B*). In another dog a focal area of atypical hyperplasia was observed in the thyroid. No evidence of hyperplasia or neoplasia was observed in any other animal. One female dog had an intraductal papilloma of the breast.

Following total thyroidectomy in the dog, vacuolated, hypertrophied "thyroidectomy cells" in the anterior pituitary gland [1] have been reported, but their origin has not been determined with certainty. Similar cells were found in the pituitary glands from all of our animals, and we, too, were unable to determine satisfactorily the cell of derivation. Plate VII*A* and *B* shows pituitaries from normal and from thyroidectomized dogs, respectively. In the rodent the thyroidectomy cell has been considered as occurring prior to the formation of a neoplasm in the pituitary gland. No pituitary neoplasm was found in any of our dogs.

The skin from a number of areas in the myxedematous dogs was examined. In those regions free from inflammation, atrophy of epidermis was pronounced. More striking than this was the appearance of the dermis in all cases (Plate VIII*A* and *B*). With the hematoxylin and eosin stain there seemed to be wide separation of the connective tissue components in both the papillary and the reticular layer of the dermis. This space

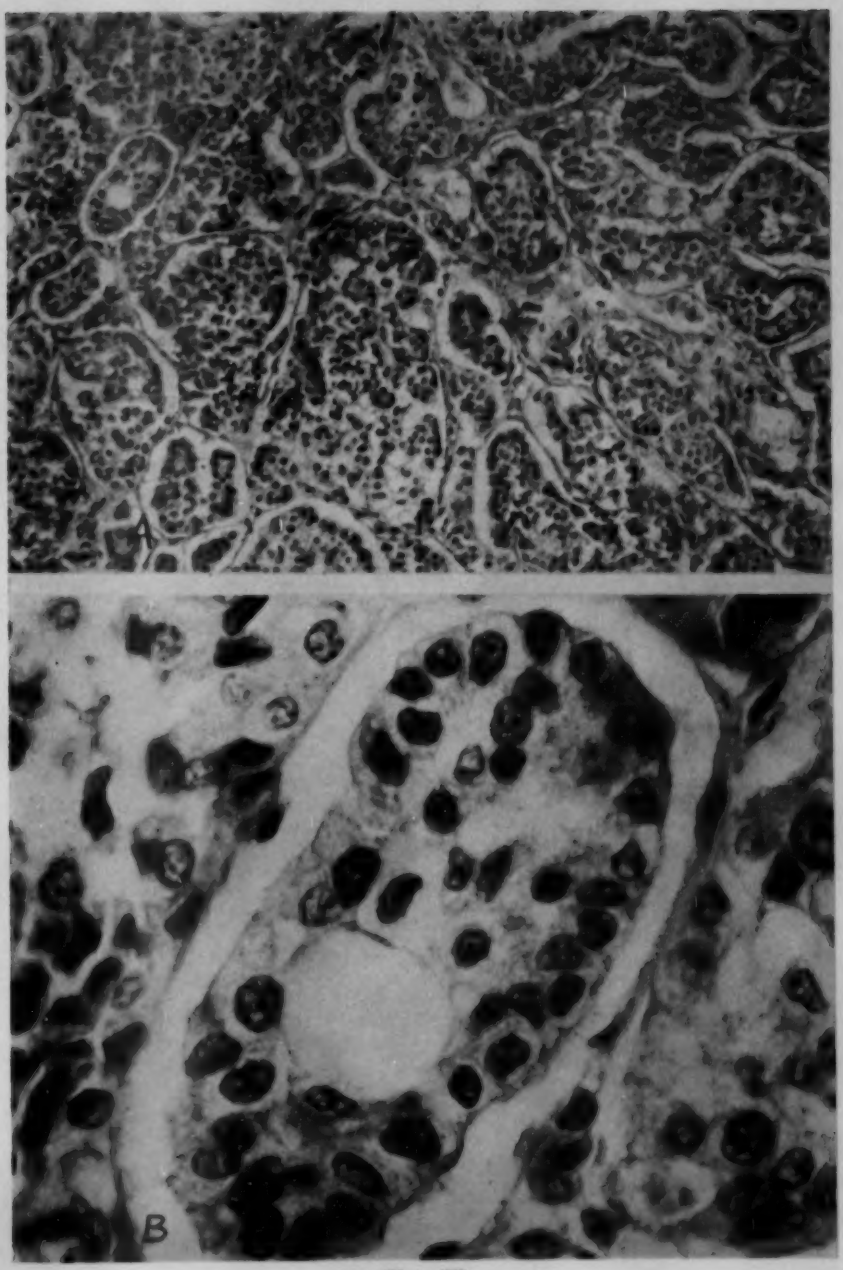


Plate VI

A, carcinoma of thyroid from same animal as that shown in Plate 5; × 80. *B*, from same animal as that shown in *A*; × 520.

was shown to be filled to a large extent with mucopolysaccharide. The edematous portion of the skin, therefore, appears to contain extracellular fluid and mucopolysaccharide. (There may be other chemical components, too.) After observing this in the dog, skin from a patient with myxedema was examined and compared with normal skin. Like the myxedematous dog, the skin of the myxedematous patient contained abundant amounts of mucopolysaccharide in the dermis.

The myocardium of both ventricles disclosed the presence of fatty infiltration, both surrounding branches of coronary arteries and between some groups of muscle fibers. The extent of this fatty change appeared to be greater than might be expected in a group of untreated mongrel dogs. No degeneration of muscle fibers was noted. The aorta in all dogs appeared essentially normal and did not show degenerative alterations, such as have been described for man and animals [15-16] in myxedema. Only minimal foci of fat cells were observed in the liver of our dogs, whereas in radiothyroidectomized dogs[1] fatty infiltration was found to be extensive. The livers of our animals still contained large amounts of glycogen.

In myxedema of various species, including man, pathologic alterations have been described which were entirely absent in our experimental group. Such reported changes (chiefly degenerative) were observed in the adrenal,[17] trachea,[18] gonads,[19] joints,[20] salivary glands,[21] and bone marrow.[22] The difference in species studied, methods of producing hypothyroidism, and time of autopsy followed in production of myxedema may have been important factors related to the changes reported by others and found absent in our dogs.

Comment

The negligible antemortem thyroid-area I^{131} uptake, the autoradiographic studies, the microscopic appearance of the irradiated thyroid tissue, and the presence of clinical myxedema are indicative of virtually complete anatomical and functional destruction of the thyroid in all our animals. The failure of many investigators to induce myxedema in the dog most probably results from the well-known difficulty in performing a really complete surgical thyroidectomy and the well-documented dependence of the completeness of the block produced by antithyroid drugs on drug dosage and frequency of administration.[23]

Our study confirms the observation of Goldberg and Chaikoff[1] that radiothyroidectomy with I^{131} in dogs results in the production of myxedema. The total I^{131} dose employed by them was considerably greater than the doses employed in this study and ranged from 0.2-3.0 mc/kg. (Table 1). Incomplete thyroid destruction was noted in some of their animals receiving as much as 3 mc/kg. in divided doses and killed 12 months later. Unfortunately, no data which would permit estimation of radiation dosage to the glands of their animals are given, and in none of our animals was incomplete destruction noted. Accordingly, consideration of both groups of animals does not permit even a crude estimation of the minimum radiation dosage required to produce virtually complete thyroid destruction in the dog. However, it is probably the increased thyroid uptake induced in our animals produced by fasting that made possible ablation with relatively low I^{131} dosage. The ease with which fasting can be carried out, the reduction in I^{131} dosage which it makes possible, and the increase in proportion of animals rendered myxedematous would appear to make this approach a desirable one in routine radiothyroidectomy in the dog.

Our crude estimates of thyroid radiation dosage (Table 2) indicate that complete destruction can be effected with a minimum dose which is probably less than 50,000 rep. Gorbman[19] estimated the thyroid lethal dose in the mouse as 25,000-30,000 rep when beta-particle loss from the gland was taken into consideration. Freedberg et al.[24] have reported three euthyroid patients in whom myxedema was induced by 29,000-44,000 rep. Two patients [25] with permanent

hypometabolism induced by 40,500 and 115,000 rep, respectively, of I^{131} showed a few remaining thyroid cells at autopsy, 29 and 5 months later, respectively. Hamilton [26] reported complete radiothyroidectomy in a dog given 0.3 mc/kg. of I^{131}, producing an estimated thyroid radiation dosage of 15,000-25,000 r.

In view of the multiple factors which enter into isotopic thyroid radiation computation and the extreme susceptibility to experimental error of most of them, the similarity of these cited figures is noteworthy. It would seem fair to say from these considerations that the radiation dosage required to produce thyroid ablation in the dog probably does not differ markedly from the dose required in man and in the mouse.

The observations of Goldberg and Chaikoff[1] that radiothyroidectomy with I^{131} in dogs results in the production of myxedema are corroborated by our results. They reaffirm the fact that the thyroid gland in the dog is functionally important in regard to certain aspects of the dog's metabolism. The pathologic alterations noted in our animals were similar to those observed by Goldberg and Chaikoff except that we did not find hepatic fatty changes (except a minimal amount in one animal). We also extended our studies beyond theirs to include nervous system, bone marrow, bone, and joint, all of which were normal. In man, however, Follis [27] states that in the hypothyroid state anatomical evidences of disturbance in the skeletal system occur.

The degenerative alterations in the anterior pituitary are of interest. Presumably they are not a direct result of radiation but are secondary to the disturbance in thyroid function. It is known that in myxedematous man mild pituitary changes may develop.

In some species in hypothyroidism, anterior pituitary neoplasms may arise. According to Gorbman,[18,28] at least five different factors have been reported as effective in rats and mice in bringing about tumorous hypophyseal enlargement: (*a*) old age, (*b*) hypothyroidism (with or without radiation), (*c*) gonadectomy, (*d*) chronic estrogen administration, and (*e*) whole-body irradiation. Various methods have been used to produce the hypothyroid state: (*a*) low-iodine diets, (*b*) feeding of goitrogenic drugs, (*c*) surgical thyroidectomy, and (*d*) radiothyroidectomy (using I^{131}). Gorbman observed pituitary tumors in mice fed Purina Laboratory Chow diet and given 200μc of I^{131}, whereas Furth states that on a similar diet with as low a dose as 25μc of I^{131} a fairly high proportion of pituitary tumors was produced. The latter occurred regularly in those animals living 13 months or longer after treatment. The neoplasms were reported to be of chromophobe origin.[29] It is of interest that in the mouse following I^{131}, anterior pituitary neoplasms develop, whereas in dogs (both Goldberg's and ours) the lesion developing in the anterior pituitary is purely degenerative in character.

In 7 of 25 male Long-Evans rats [30] that had received single intraperitoneal injections of 400μc of I^{131}, carcinoma of the thyroid developed one and one-half to two years later. Metastases were found in five of the seven rats with thyroid carcinoma. The areas invaded by the metastases were lung, lymph node, bone, adrenal gland, and subcutaneous tissue. In Chaikoff's laboratory, in over 1,000 rats up to two and one-half years of age, no spontaneous malignancy of the thyroid was found. Bullock and Curtis [31] noted that "of the many thousands of rats which have been examined post mortem, only two developed thyroid tumors. One was a benign adenoma, the other, a malignant growth in a 948-day-old rat."

In another laboratory,[32] the carcinogenic potency on the thyroid of 5μc, 30μc, and 100μc of I^{131} was tested on a total of 100 rats (Lister strain) alone and combined with a subsequent 15-month course of methylthiouracil. The radioactive iodine was found to increase the formation of thyroid adenomas as compared with controls in the 5μc and 30μc groups but not in the 100μc group. Out of 20 rats treated with a combination of 30μc of I^{131} and methylthioura-

cil, 5 developed thyroid carcinomas. The radiation dosage range to the thyroid of 2,270 to 16,200 rep in the latter experiment is likely to include the dosage aimed at, of about 9000 rep in the treatment of toxic diffuse goiter (Graves' disease).

According to Chapman and associates,[33] I^{131} radiation produces fibrosis and cellular damage in the thyroid, resulting in bizarre nuclear forms; yet some of the remaining follicles appear hyperplastic. Microscopic sections from 44 thyroids studied after irradiation revealed no evidence of malignancy. In their series of 585 patients treated during the past 12 years (beginning in 1942), no known carcinoma of the thyroid attributable to radioactive iodine has developed.

In our experiments on eight dogs in which myxedema was produced with I^{131}, one animal developed an area of atypical hyperplasia in the thyroid, and another animal had a carcinoma of the thyroid. While these are two interesting observations, the carcinoma cannot specifically be attributed to the radiation, since thyroid malignancy is not uncommon in the dog.[34] However, our animals were killed 14½ months after treatment, and it is conceivable that neoplasms could have developed in the thyroid had the dogs been allowed to live out their life spans. Both the frequency of occurrence of carcinoma of the thyroid in a given species and the time at which neoplasms occur in a given species are important considerations in evaluating this problem. In regard to this, carcinoma of the thyroid does occur spontaneously in the various species mentioned in this report, apparently in this order of frequency, from least to most—rat[31] mouse,[35] dog,[34] man. As to the time at which neoplasms occur in these species following radiation, Brues[36] has constructed an interesting table between life span and minimum latent period at which a neoplasm may develop. In his table the suggested factors are as follows: for life span—1 for rat and mouse, 5 for the dog, and 35 for man, the induction periods being, respectively, 1, 3, and 8 for these species. It is therefore conceivable that, since carcinoma of the thyroid has occurred in rats 1.5 to 2 years following I^{131}, carcinoma might have developed had our dogs lived longer, and it may very well require eight years or more longer before the possibility of carcinoma of the thyroid arising in patients with hyperthyroidism treated with I^{131} can be settled. As Brues has further pointed out: "It is, of course, interesting to know whether the latent period may be a function of life span of the host, or of biological rather than physical time."

The possibility that I^{131} treatment may produce carcinoma of the thyroid rests on only one positive result.[30] Other extensive and carefully done experiments[37] have failed to demonstrate any increased incidence of thyroid carcinoma following I^{131}. In fact, the experiments of Goldberg and Chaikoff[30] have not been confirmed in our laboratory, even when their experiments were repeated exactly as to amount of I^{131}, course of study, and strain of rat.[38] It should be pointed out, we believe, that, although the possibility that I^{131} is carcinogenic to the thyroid cannot be excluded, it has been equally difficult to demonstrate that I^{131} will produce cancer. The importance of the possibility, however, does require that studies of the injurious effects of I^{131} should be continued and their results reported even if the results are negative.

Summary

Eight mongrel dogs were given a single dose by mouth of I^{131} ranging from 0.063 to 0.98 mc. per kilogram of body weight. All dogs developed myxedema. Clinically, this was characterized by decrease in food consumption, increase in body weight, lethargy, hyperkeratosis and epilation of skin, hyperglobulinemia, and hypercholesteremia. Pathologically, atrophy of thyroid follicles resulted in virtually complete ablation of the gland in all dogs. Atrophy of skin and edema of corium developed. Cellular degeneration occurred in a large part of each anterior pituitary gland. A single

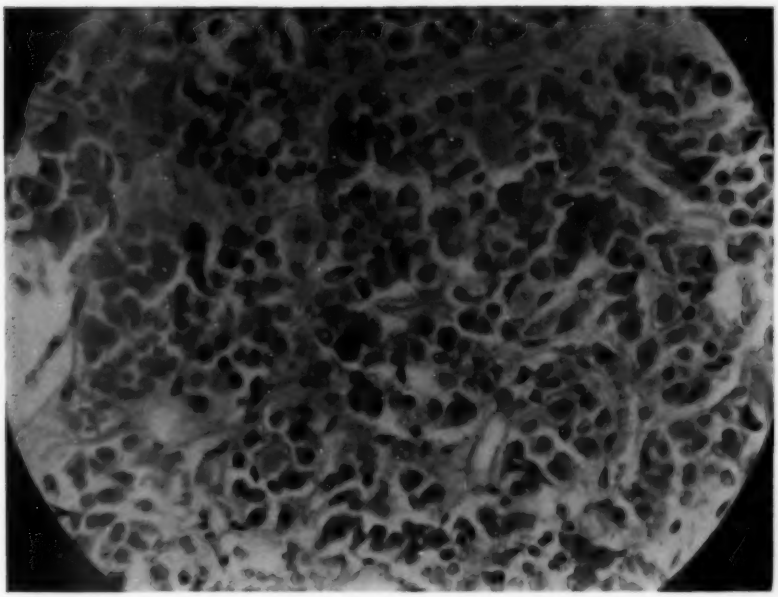


Plate VIIA

(A) Normal anterior pituitary in adult dog.

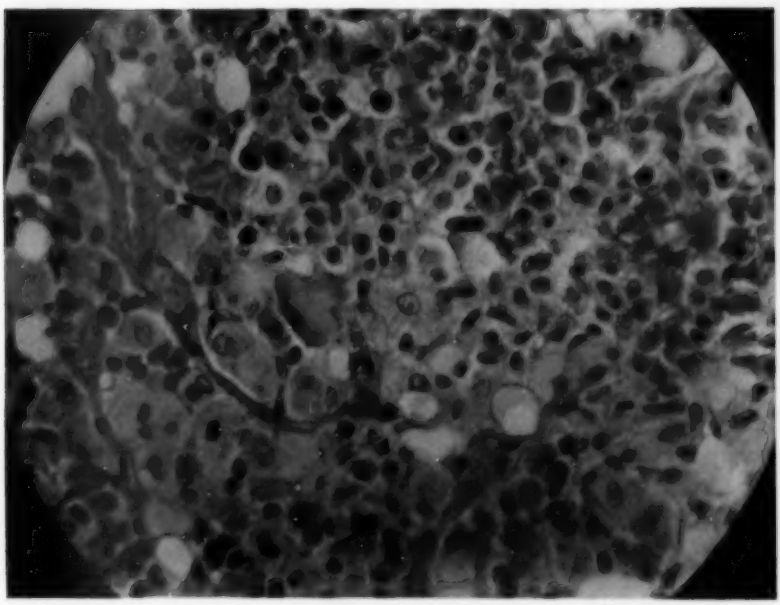


Plate VIIB

(B) Anterior pituitary in radiothyroidectomized dog.

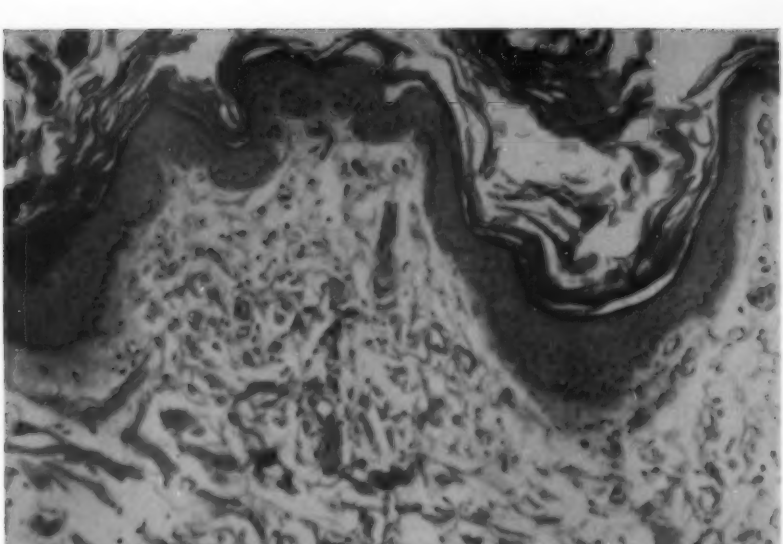


Plate VIIIA

(A) Skin from myxedematous dog.

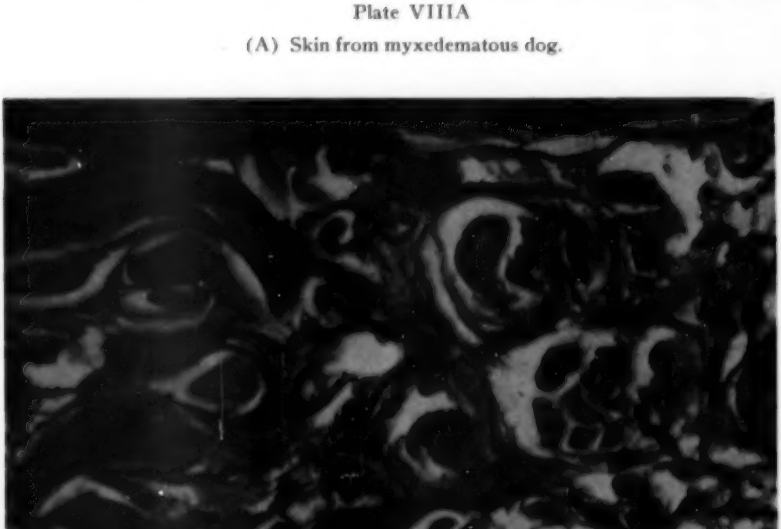


Plate VIIIB

(B) Same as A, acid mucosaccharide. × 320.

dog had a carcinoma of the thyroid which probably bears no relation to the therapy, since the other seven animals did not have thyroid carcinoma.

Appendix*

The following terms are defined:

q_{it}=radioactivity (fraction of administered dose) in Compartment i at time t
λ_{ir}=fraction of q_i transferred to Compartment r per day
A_{ji}=coefficient (ordinate intercept value of the component) of the ith exponential term in the equation relating radioactivity to time in Compartment j
λ_{ii}=sum of all fractional rates of loss of radioactivity from Compartment i
α_i=exponential constant of the ith component
K_{1e}=fraction of iodide in the iodide space excreted by the kidneys per day
K_{1t}=fraction of iodide in the iodide space entering the thyroid per day
t=elapsed time, in days
A_{44}=asymptotic value of cumulative urinary excretion of radioactivity
A_{33}=thyroid content of radioactivity at the beginning of the discharge phase
λ_{n0}=fraction of q_n lost by nonrenal excretion per day
It=radioactivity in the iodide space at time t
D_β=cumulative beta radiation dosage, in reps
Ct=concentration of radioactivity in microcuries per gram at time t
$\overline{E}_\beta$=mean beta energy, in mevs
T.W.=estimated thyroid weight, in grams
λ_d=physical decay constant of I^{131} (fraction per day)
μc_s=administered radioactivity, in microcuries.

* Prepared by C. Lewallen.

To estimate the radiation dose, a thyroid "uptake phase" (zero time to three days) and a "discharge phase" (subsequent to the third day) were assumed. Hereafter the thyroid will be designated Compartment 1, Urine, Compartment 4, and the compartments of organic iodine receiving labeled material from the thyroid will be designated Compartments 2 and 3. Thyroid weight was estimated from the data of Mulligan and Francis.[39]

Discharge Phase.—The urinary excretion data were plotted on semilogarithmic coordinates, with elapsed time computed from the time of resumption of the diet as zero time (third day following administration of the dose). Values of A_{44} were chosen empirically such that the terminal portion of the plot of $A_{44}-q_{4t}$ described a straight line. The additional components were obtained by serial subtraction in the conventional way. The half-time ($T_{\frac{1}{2}}$) for each component was determined by graphical inspection, and the corresponding value of α obtained from the relationship $\alpha=0.693/T_{\frac{1}{2}}$. The coefficients were obtained from the ordinate intercept values of the components. An illustrative plot is shown in Figure 2. Three exponential components were obtained in the case of six dogs, and two components in the other two (not including A_{44} as a component). With three components at hand, cumulative urinary excretion can be related to time in the form:

$$q_{4t}=A_{41}e^{-\alpha_1 t}+A_{42}e^{-\alpha_2 t}+A_{43}e^{-\alpha_3 t}+A_{44} \quad (1)$$

From the findings of other investigators cited in the text, it is reasonable to consider the thyroid in the discharge phase as a single compartment releasing radioactivity at a single fractional rate (without reuptake of released isotope) to at least two compartments of organic iodine having different rates of loss. The presence of three components in the excretion data is also indicative of a three-compartment system. Accordingly, the excretion data were analyzed with reference to the three-compartment unidirectional flow model shown in Figure 3*A*. The rate constants λ_{20} and λ_{30} are included to provide for nonrenal excretion, such as fecal. The solution of the parallel model (Fig. 3*A*) follows.

Neglecting the iodide compartment, it is apparent that

$$dq_1/dt=-q_1\lambda_{12}-q_1\lambda_{13}=-q_1\lambda_{11} \quad (2)$$
$$dq_2/dt=q_1\lambda_{12}-q_2\lambda_{20} \quad (3)$$
$$dq_3/dt=q_1\lambda_{13}-q_3\lambda_{30} \quad (4)$$
$$q_{4t}=\lambda_{24}\int_0^t q_2dt+\lambda_{34}\int_0^t q_3dt \quad (5)$$

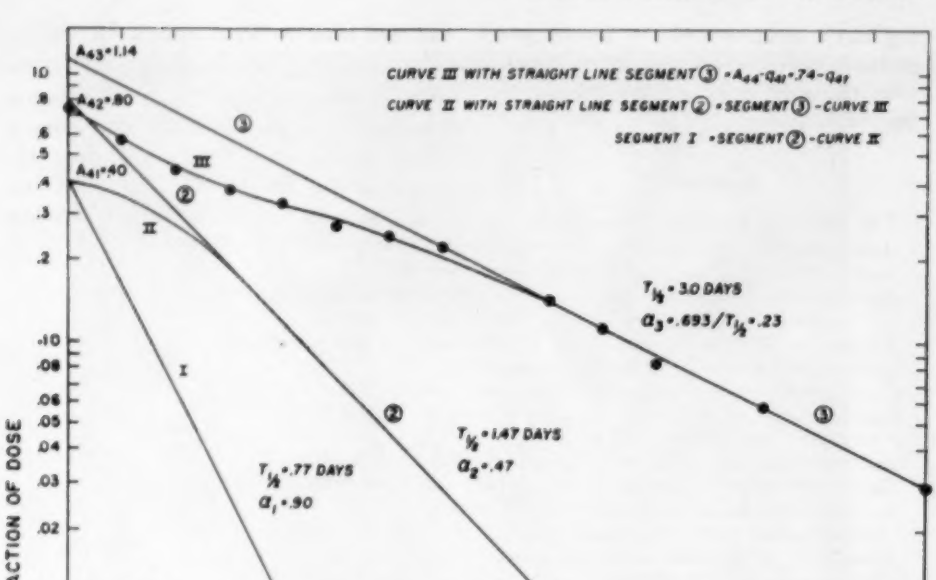


Fig. 2 (Dog A-61).—Components of urinary excretion curve of I^{131} following administration of a thyroid ablation dose.

By standard methods [40] the solution of these differential equations gives

$$q_{11}=A_{11}e^{-\lambda_{11}t} \quad (6)$$

$$q_{21}=A_{21}e^{-\lambda_{11}t}-A_{22}e^{-\lambda_{22}t} \quad (7)$$

$$q_{31}=A_{31}e^{-\lambda_{11}t}-A_{32}e^{-\lambda_{22}t} \quad (8)$$

$$q_{41}=A_{41}e^{-\lambda_{11}t}+A_{42}e^{-\lambda_{22}t}+A_{43}e^{-\lambda_{33}t}+A_{44} \quad (9)$$

where

$$A_{21}=\frac{A_{11}\lambda_{12}}{\lambda_{22}-\lambda_{11}},\ A_{31}=\frac{A_{11}\lambda_{13}}{\lambda_{33}-\lambda_{11}}$$

$$A_{41}=\frac{-A_{21}\lambda_{24}-A_{31}\lambda_{34}}{\lambda_{11}},\ A_{42}=\frac{A_{21}\lambda_{24}}{\lambda_{22}},\ A_{43}=\frac{A_{31}\lambda_{34}}{\lambda_{33}}$$

$$A_{44}=-A_{41}-A_{42}-A_{43}$$

If at the beginning of the discharge phase radioactive iodide of quantity I_o is present which has not entered the thyroid system, this will be excreted at the rate K_{1o}, and the quantity in the urine at time t will be $I_o-I_oe^{-k_{1o}t}$. This will summate with activity from Compartments 2 and 3 and Equation 9 becomes

$$q_{41}=A_{41}e^{-\lambda_{11}t}+A_{42}e^{-\lambda_{22}t}+A_{43}e^{-\lambda_{33}t}-I_oe^{-k_{1o}t}+A_{44}+I_o \quad (10)$$

Similarly, solution of the series model shown in Figure 3*B* gives an equation of q_{41} identical to Equations 9 and 10, except that the expressions for the coefficients A_{41} are different. The identity of form of Equations 1 and 9 should be noted. The solution shows that in a system of this type λ_{11}, λ_{22}, and λ_{33} appear as the exponential constants (α's) of the components of q_{41}, the urinary excretion curve, as does also K_{1o}, if radioactive iodide is present at zero time. (The identity of the α_1's with the λ_{11}'s does not hold in more complex systems.[41]) The values of the α's obtained are shown in Table 2. The signs of the coefficients A_{41}, A_{42}, and A_{43} were, respectively, negative, positive, and negative. Iodide was dismissed

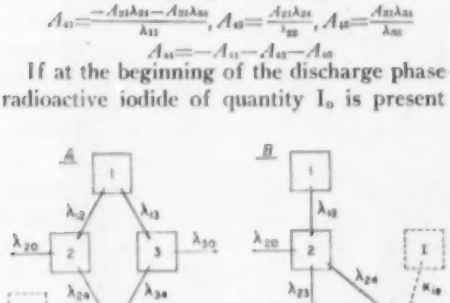


Fig. 3.—Diagrams of kinetic models used for analysis.

from consideration, since the α agreeing most closely with K_{1e}, as determined on the tracer studies (α_2), was associated with a coefficient of positive sign (Equation 10). Accordingly, A_{11} was computed as the total retention of isotope at the time of resumption of the diet.

Although it was known that the rate of loss of radioactivity from the thyroid λ_{11} was represented by one of the α's obtained from the excretion curve, no reliable method of choosing the correct one was evident. Consequently, radiation dosage during the discharge phase was computed with $\lambda_{11}=\alpha_1$ and $\lambda_{11}=\alpha_3$, thus obtaining the possible range of radiation dosage in this phase.

It can be readily shown [42] that the beta radiation dose rate from a beta-emitting isotope uniformly distributed in an absorbing medium is given by the equation

$$\frac{dD_\beta}{dt}=55C_t\overline{E}_\beta g \text{ reps per day} \qquad (11)$$

By integration

$$D_\beta=55\overline{E}_\beta\int_0^t C_t dt=11\int_0^t C_t dt, \qquad (12)$$

accepting a value of 1.0 for g and 0.20 for E_β.[42] In the discharge phase $q_1=A_{11}e^{-\lambda_{11}t}$ (Equation 6), where t is the elapsed time after resumption of the diet, which was three days after administration of radioactivity. Accordingly

$$C_t=\frac{A_{11}e^{-\lambda_{11}t}\mu c_0 e^{-\lambda_d(t+3)}}{T.\,W.}=\frac{\mu c_0 A_{11}e^{-3\lambda_d}e^{-(\lambda_{11}+\lambda_d)t}}{T.\,W.} \qquad (13)$$

$$D_\beta=11\int_0^\infty C_t dt=\frac{11\mu c_0 A_{11}e^{-3\lambda_d}}{(T.\,W.)(\lambda_{11}+\lambda_d)} \qquad (14)$$

Uptake Phase.—It is known that urinary excretion and thyroid uptake account for virtually all administered iodide and that during the first few days thyroid uptake as a good approximation can be regarded as a unidirectional process.[6] If the administered dose is unity, then

$$I_t=e^{-(K_{1e}+K_{1g})t} \qquad (15)$$

$$q_{11}=K_{1g}\int_0^t e^{-(K_{1e}+K_{1g})t}dt \qquad (16)$$

$$=\frac{K_{1g}}{K_{1g}+K_{1e}}\left[1-e^{-(K_{1g}+K_{1e})t}\right]$$

For small values of K_{1e}, Equation 16 reduces to

$$q_{11}\cong 1-e^{-K_{1g}t} \qquad (17)$$

In view of the small values of urinary excretion in the three days following administration of the dose, Equation 17 was

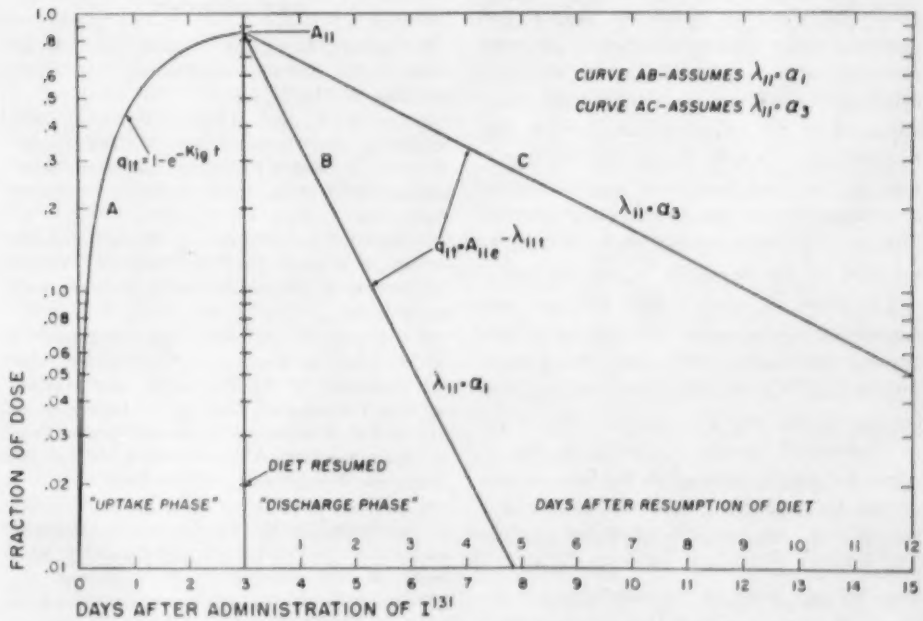


Fig. 4 (Dog A-61).—Curves of computed gland content of isotope with time.

solved for K_{1g}, equating q_1 at three days to A_{11}.

$$C_1=\frac{\beta C_0 e^{-\lambda_1 t^1}}{I \cdot W \cdot}(1-e^{-K_{1g}t^1}) \qquad (18)$$

$$D_\beta=11\int_0^3 C_1 dt=\frac{11\beta c_0}{I \cdot W \cdot}$$

$$\left[\frac{1-e^{-3\lambda_3}}{\lambda_3}-\frac{1-e^{-3(\lambda_3+K_{1g})}}{\lambda_3+K_{1g}}\right] \qquad (19)$$

Figure 4 shows the computed time course of isotope in the thyroid for one of the animals in both the uptake and the discharge phase. Curve A_e assumes $\lambda_{11}=\alpha_3$ and curve AB assumes $\lambda_{11}=\alpha_1$. Equation 14 was evaluated for $\lambda_{11}=\alpha_1$ and $\lambda_{11}=\alpha_3$ and the values alternately added to the evaluation of Equation 19, giving the range of radiation dosage shown in Table 2.

It is evident that this approach leans heavily on the assumption that the discharge of isotope from the glands due to radiation damage does not begin before 72 hours. Although this is probably a reasonable assumption for the low-dose animals, discharge prior to this time may have occurred in the high-dose animals, such that the computed radiation dosages in the uptake phase are in error by overestimation. However, one would expect that if discharge of organic iodine of a significant degree had occurred prior to reestablishment of renal capacity to excrete iodide at 72 hours, a detectable iodide component would have appeared in the excretion data. Such was not the case. Despite its obvious shortcomings, we feel that this kinetic approach gives a truer picture of the actual gland irradiation than the assumption that all activity retained by the animal is in the thyroid.

The gland of mean weight 1.56 gm. was considered to consist of two spheres of unit density and radius 0.59 cm. The gamma contribution to the dose was disregarded because of the negligible gamma absorption in a sphere of this size. In media of dimensions not greatly exceeding the path length of the beta particles, there is discrepancy between the beta energy liberated and the beta energy absorbed. The beta radiation dose at any point in the medium can be shown to be proportional to a geometry factor g, which is an evaluation of a complex integral. To evaluate g, only the 0.605 mev beta which provides 94% of the beta energy of I^{131} was considered, and a value of 18 for the linear absorption coefficient μ was accepted.[44] Substituting the values for radius and μ in the table by Oddie [42] gave the following values for g at various points in the sphere (hemithyroid): center, 1.0; central 78% of the sphere volume, 1.0-0.91; outer 22% of the sphere volume, 0.91-0.48. The dosages given in Table 2 are based on a value of g of 1.0 and apply only to the center of the hemithyroids. The doses toward the periphery are less, as indicated by the above values of g.

Radiation dosage here was expressed in reps for purposes of ease of comparison with cited figures in the literature. Reps may be converted to rads (the currently favored unit) by multiplying by 0.93.[45]

Drs. J. E. Rall and M. Berman allowed us to examine their unpublished data on radiothyroidectomy in man. Mrs. Helen Hamel gave technical assistance.

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Comparison of Acute Pathological Effects of Alpha- and Beta-Particle Radiation of Rat Thyroid

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The selective localization by the thyroid gland of radioactive astatine (At^{211}), element 85, an alpha-particle emitter, with a half-life of 7.3 hours, and of radioactive iodine (I^{132}), element 53, a beta-particle emitter, with a half-life of 2.3 hours, was utilized to compare the functional and histopathological effects of these two types of radiation following delivery of known dosages of these isotopes. Such a study should be of importance in determining whether the effects are related to the type of radiation given or are correlated quantitatively in terms of equivalent energy release from each isotope.

Although there is no complete theory of the relative biological effectiveness (RBE) of particles with different ionization patterns, those particles with high linear energy

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Plate I
Autoradiograph of normal rat thyroid gland administered At^{211}; × 512.

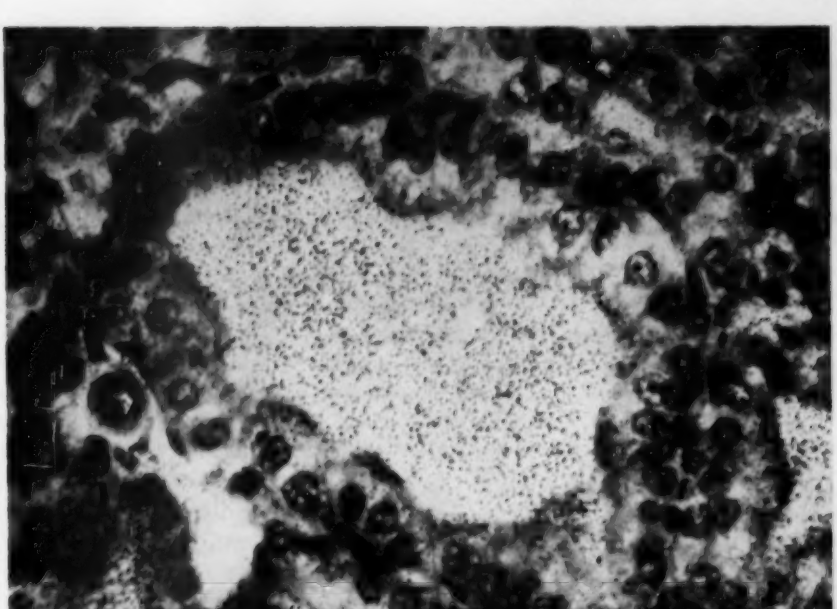


Plate II
Autoradiograph of normal rat thyroid gland administered I^{131}; × 512.

transfer (LET) tend to have higher relative biological effectiveness.[1] The linear energy transfer rate is much greater for the heavy particle (alpha) than it is for the beta particle. Thus, the alpha-particle track will be characterized by being short, dense, and with many ionizations per unit distance, whereas the beta-particle track will be longer, more tortuous, and with a relatively sparse number of ionizations per unit of path length. The alpha and beta tracks are shown with radioautographs of normal thyroid gland following administration of At^{211} (Plate I) and I^{131} (Plate II). However, in the case of I^{132} (beta) particles their less dense ionization exposes only single grains of the emulsion, and tracks are not found very often. In actual practice, only the densely ionizing At^{211} (alpha) particle, as it traverses the film emulsion over the At^{211}-containing thyroid tissue, exposes successive grains, so that a track can be seen.

Materials and Methods

The methods of the production of At^{211} and I^{132}, administration, and calculation of dose to the gland have been reported previously [2] and will be presented here only briefly. Male Sprague-Dawley rats maintained on an iodine-poor diet were administered a tracer amount of At^{211}, or 11μc, 22μc, or 44μc of At^{211}, or a tracer amount of I^{132}, or 262μc, 525μc, or 1050μc of I^{132}. The animals receiving the tracer doses were killed at convenient intervals during the first 24 hours following isotope administration, and the isotope content of the thyroid gland was determined to calculate the radiation dose to the gland. The remaining animals received a tracer dose of I^{131} four days after the initial administration and were killed 18 hours after the administration of I^{131}. The I^{131} content of the glands was determined and the thyroid glands were prepared routinely for microscopic examination.

Pathology

The thyroid gland of a typical control rat is shown in Plate III. The former shows both lobes and isthmus. The peripheral follicles are notably larger than those in the



Plate III

Normal rat thyroid gland. × 35.

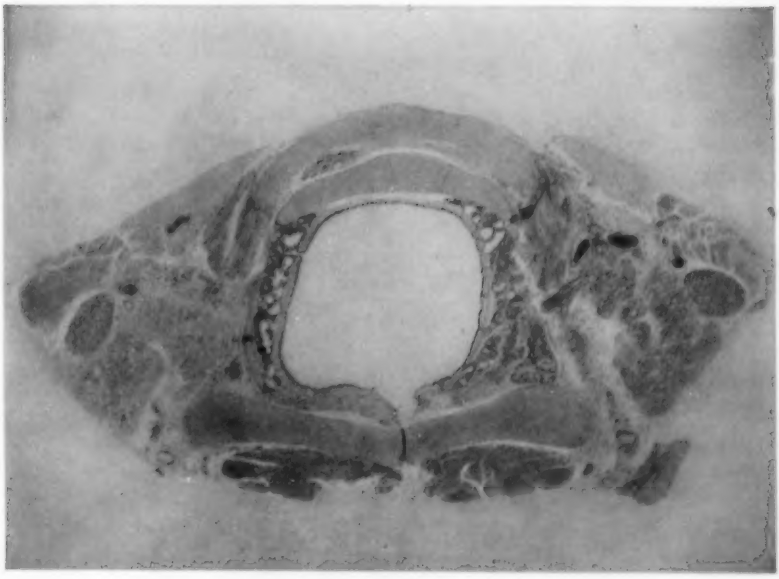


Plate IV

Rat thyroid gland five days after administration of 22μc At^{211}. × 35.

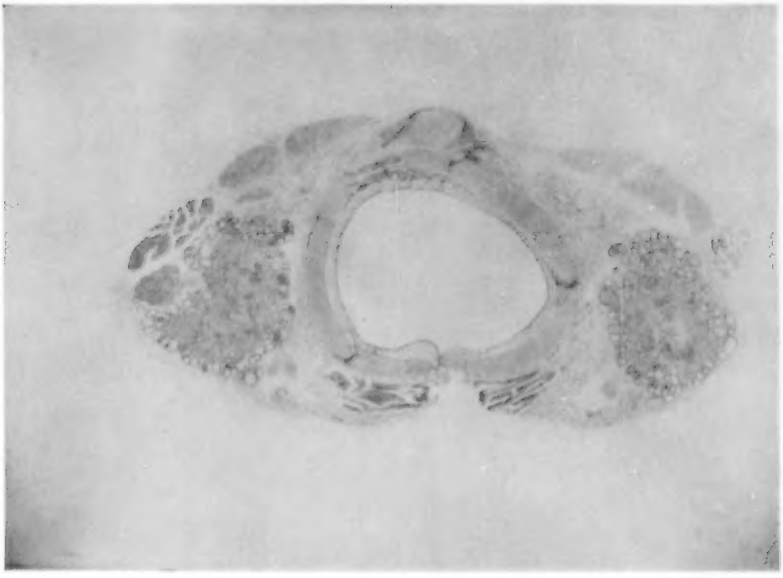


Plate V

Rat thyroid gland five days after administration of 1050μc I^{130}. × 35.

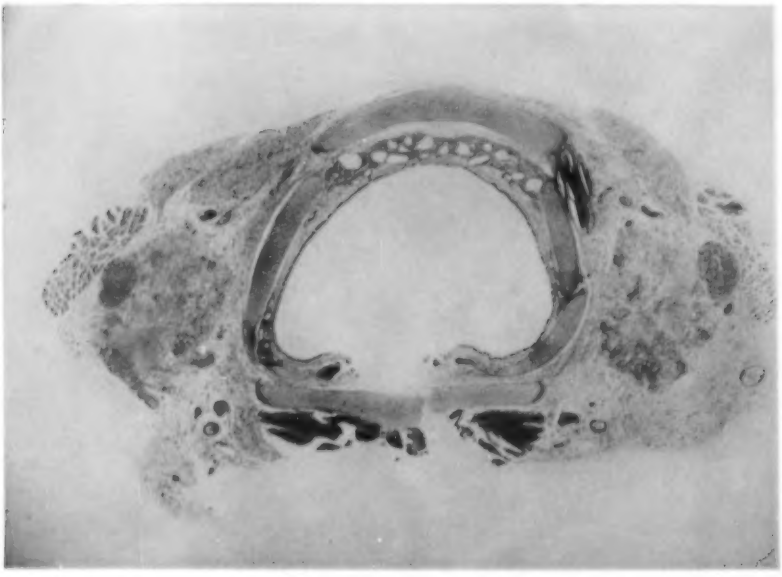


Plate VI

Rat thyroid gland five days after administration of 44μc At^{211}. × 35.

center of the lobe, being in some instances as much as five times as large. Under the high-power magnification, the follicular epithelial cells appear round to oval, with their nuclei lying in the basal portion of the cells. The cytoplasm is abundant. All follicles tend to abut against one another, so that intervening connective tissue is sparse. Many capillaries are present. Colloid, in varying amounts, is found in nearly all follicles.

The animals receiving 262μc of I^{132} disclosed no morphologic alterations in the thyroid. Occasionally with 525μc of I^{132}, and frequently with 11μc of At^{211} stromal edema was observed. No vascular or follicular epithelial changes were found in these thyroids, with the exception of two cases. Most of the rats getting a dose of 22μc of At^{211} showed, in addition to stromal edema, early evidence of epithelial degeneration in follicles scattered throughout the gland. This observation is shown in Plate IV. Here no completely normal follicle can be found. In some instances individual epithelial cells appear intact, while the remainder are in varying stages of degeneration. It is difficult at this dosage to be certain whether true morphologic changes can be detected in the arterioles. Occasionally the impression gained is that at least minimal necrosis is developing in the media.

In the highest doses of both I^{132} and At^{211} the destructive changes become quite striking. With 1050μc of I^{132} irradiation damage is not uniform; the maximal changes occurring are demonstrated in Plate V. The central portion of the gland is completely necrotic, whereas the peripheral rim of follicles appears essentially normal. The parathyroid is also normal. With 44μc of At^{211} (Plate VI), the entire thyroid looks necrotic, none of the cellular debris being of recognizable origin. Intermingled are focal areas of hemorrhage, resulting from the acute vasculitis of arterioles and disruption of capillaries. The connective tissue surrounding the necrotic follicles is edematous. The adjacent trachea and its mucosal lining are intact. The parathyroid appears normal.

TABLE 1.—*Method of Grading Lesions Produced by At^{211} and I^{131}*

Grade	
0	Normal
I	Edema of stroma only
II	Edema of stroma and early evidence of epithelial degeneration in acini
III	Marked central acinar necrosis; intact peripheral acini
IV	Complete acinar necrosis

After studying the thyroid glands microscopically, an attempt was made to grade the lesions observed. This, of necessity, is somewhat arbitrary but is helpful in attempting to compare the irradiation damage at the various doses given with both isotopes. Table 1 shows the method of grading the lesions. The results of grading the lesions are presented in Table 2 and discussed under the section on "Correlation."

Correlation

The amount of isotope administered, the radiation dose delivered to the gland by this amount of isotope, the per cent inhibition of thyroidal accumulation of I^{131}, and the pathological grade(s) of the glands are presented in Table 2. It can be seen that as the radiation dose delivered to the gland increased, the function of the gland was increasingly impaired and the severity of pathological lesion also increased.

TABLE 2.—*Functional and Pathological Effects of At^{211} or I^{132} Administration on Rat Thyroid Studied Five Days After Initial Administration of Isotopes*

Amount and Type of Isotope Administered		Dose, Rep	Per Cent Inhibition of I^{131} Uptake	Grade of Lesions				
				0	I	II	III	IV
None		None	None	10				
262μc	I^{132}	15,000	6	5				
525μc	I^{132}	30,000	32	8	2			
11μc	At^{211}	35,000	92		8	2		
1050μc	I^{132}	60,000	75		3	2	5	
22μc	At^{211}	70,000	99		2	7	1	
44μc	At^{211}	140,000	100					10

It is apparent that the destructive action of both particles can be correlated with the amount of radiation dose delivered to the gland. This is particularly true of the observed pathological lesions; i. e., the dose determines the damage regardless of the type of particle that produced the radiation dose. However, when only the functional aspects of the radiation dose were considered, a slightly different result was obtained. First, within the doses resulting from a particular particle, the larger the dose the more impairment of function, but the alpha dose gave a larger impairment than the beta dose by a factor of more than 2 at 30,000-35,000-rep range and less than 2 at the 60,000-70,000-rep range. The discrepancy between functional and pathological effects may well be only an apparent one. It is certain that the functional measurements give a more quantitative measurement of destruction than do pathological judgments of destruction. The large range of radiation doses delivered to the gland also make comparisons difficult, particularly in the At^{211}-treated animals.

In addition, in any attempted correlation of functional and pathological estimations of the over-all status of an endocrine gland, it is usually to be expected that functional changes may have occurred before these changes can be detected by observed histological changes. The fact that the functional changes and the observed pathological changes, in the present study, are in the same direction, i. e., a depression of thyroid function following irradiation, suggests that form and function are certainly correlated.

The objective of this report was to present a comparison of alpha-particle and beta-particle radiation on thyroid tissue. The destructive action of these two particles has been suggested to be qualitatively similar. However, quantitative studies are always of value, but it is felt that the data herein presented do not allow an exhaustive analysis of the possible quantitative differences between alpha- and beta-particle radiation. Some of the reasons for not attempting a further quantitative estimate have been alluded to before, e. g., the wide range of radiation doses and the difficulties involved in assessing the finer morphologic details of the pathological effects produced by the radiations. Probably even more important is the fact that the beta particle traverses a much longer path than the actual size of the rat thyroid gland. For this reason, the dose delivered to the periphery of the gland will be much less than the dose delivered to the center of the gland. Although this factor was taken into account in the calculation of the beta radiation dose, the fact remains that the dose from beta radiation is much less uniform than the dose from the alpha particles. Until a method is devised whereby a truly uniform dose is delivered to the gland from both particles, it is probably unwise to attempt to conclude definitely that alpha-particle and beta-particle radiation effects differ in a quantitative aspect.

These findings are in general agreement with the report of Lindsay et al.,[3] who noted that irradiation with I^{131}, x-rays, neutrons, and radon produced lesions that were qualitatively similar, and the less extensive studies of Hamilton et al.,[4,5] in which the destructive actions of I^{131} and At^{211} were found to be similar on the rat thyroid, and of Watts,[6] who noted that the metabolic rats than in At^{211}-treated rats, although they rate decreased more slowly in I^{131}-treated ultimately reached the same value.

Summary

The acute functional and pathological alterations in the thyroid of the rat were studied after administration of known doses of alpha (At^{211})- and beta (I^{132})-particle radiation.

1. Functional impairment increased with increments in dosage with both isotopes.

(*a*) The alpha dose gave a slightly larger impairment than the beta dose.

2. Pathologically, the degree of damage to the thyroid was not related to the type of radiation but appeared to be correlated qualitatively and quantitatively in terms of equivalent energy release from the two isotopes.

(*a*) With 525μc of I^{132} (30,000 rep) occasional stromal edema was noted in the thyroid.

(*b*) With 11μc of At^{211} (35,000 rep) stromal edema was always present, and with 22μc of At^{211} (70,000 rep) there was also follicular epithelial degeneration.

(*c*) Administration of 1050μc of I^{132} (60,000 rep) resulted in necrosis of all but a peripheral rim of follicles.

(*d*) Total destruction of all follicles occurred after 44μc of At^{211} (140,000 rep).

3. Functional and pathological changes were in the same direction, but no complete correlation of the two could be made.

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Effects of Cortisone on the Developing Chick Embryo

A Histologic Study

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Alterations of the gross anatomy of the developing chick embryo after administration of cortisone were first described by Karnofsky [1] and later corroborated by Evans.[2] The histological changes, however, have not been reported previously. In the course of study designed to elucidate the mechanism of virus-induced diseases in the embryonated egg, the influence of cortisone administration was investigated. As a necessary preliminary adjunct, a study of the effect of the hormone on the normally developing embryo was carried out. The present report describes the gross anatomical and histological alterations in embryonic development following cortisone treatment. The possible mode of hormonal action is also discussed.

Materials and Methods

Fertile White Leghorn eggs which had been incubated at 37 C for 10 days were inoculated on the chorioallantoic membrane (CAM) with cortisone acetate. The 10th day was chosen for injection because, as Karnofsky [1] has shown, cortisone does not affect the embryo until the 8th to the 10th day of development. A small perforation was made through the shell membrane over the CAM by means of an egg punch.* A hole was also placed over the air sac. Dropping of the chorioallantoic membranes was carried out by gentle suction over the air space with a rubber teat. Each embryo then received a single dose of 0.5, 1.25, 2.5, or 3.75 mg. of cortisone acetate. Treated and control embryos were killed at the end of 12, 14, and 18 days (Table). Mortality varied with the dose of cortisone and with the duration of incubation; it was greatest among the 18-day embryos injected with 3.75 mg. of cortisone acetate. Dead embryos were examined grossly, but not microscopically, because of the rapid deterioration of embryonic tissue under incubation. Gross sections were taken through the head, neck, thorax, abdomen, and chorioallantoic membranes of the viable embryos following fixation in 10% isotonic neutral formalin. Microscopic preparations were stained with hematoxylin and eosin and the periodic acid-Schiff method, and selected sections of kidney and liver were stained with Sudan IV.

Results

Gross Observations.—Administration of cortisone caused a quantitative interference in embryonic growth that varied with the dose used and the duration of the experiment. The retardation of growth was symmetrical except for the eyes, which were disproportionately large, and the liver, which was disproportionately small. This was previously noted by Evans.[2]

The CAMs were abnormally small in area, and the yolk sac did not completely develop. The body wall did not completely close, and evisceration was found even after 18 days of incubation.

The feather development was abnormal, the feathers being quite short. This was most apparent on the head, so that some embryos appeared to be almost bald. Close inspection, however, did reveal head feathers

Number of Embryos Studied in Each Group

Dose of Cortisone, Mg.	Day Killed		
	12th	14th	18th
0.5	0	4	4
1.25	4	6	6
2.5	0	6	6
3.75	4	6	6
Controls	2	4	4

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From the Department of Pathology, University of California School of Medicine, (Drs. Siegel and Gerstl), and the Laboratory Service of the Veterans Administration Hospital, Oakland, Calif. (Drs. Smith and Gerstl).

* Purchased from Virtis Company, Yonkers, N. Y.

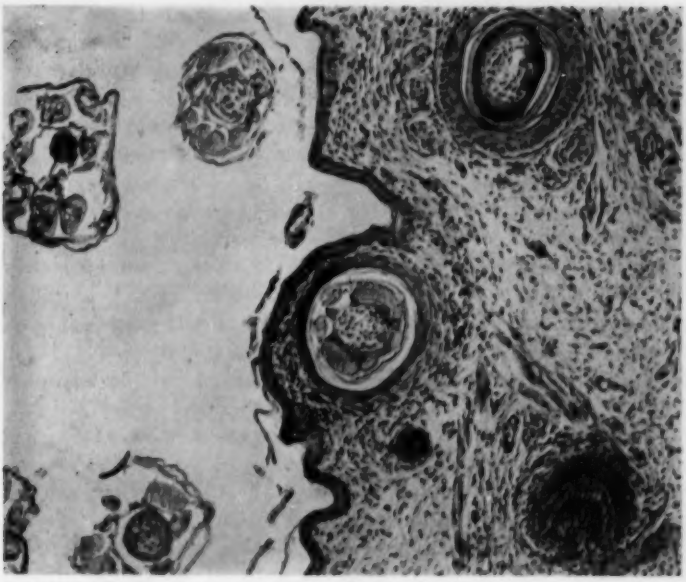


Fig. 1.—Normal skin, 18-day control. Note heavy keratin layer of feather shaft, rounded keratin barbs, serrated epithelium, and condensed medulla. Hematoxylin and eosin; × 100.

that were little more than papillae. Cortisone may have interfered with the formation of new papillae, since the number of feather buds per square centimeter was definitely less in the treated than in the control embryos.

Microscopic Observations.—1. Chorioallantoic Membrane: The CAMs were thinner than normal in the cortisone-treated embryos, as well as being smaller in area. This apparently resulted from a defect in mesoclermal development, since the epi-

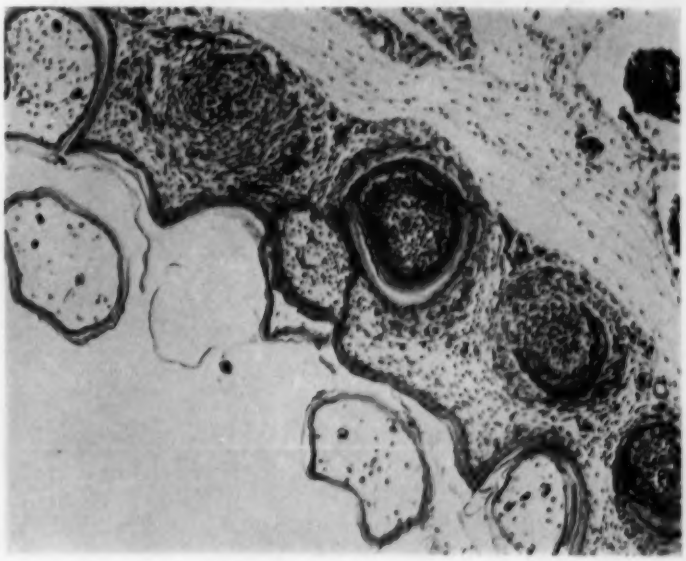


Fig. 2.—Skin, 18-day embryo treated with 3.75 mg. of cortisone acetate. Section shows dilation and hypocellularity of feather medulla, atrophy of feather epithelium, and absence of barb formation. Hematoxylin and eosin; × 100.

thelium was not altered. As suggested by Karnofsky,[1] such retardation may result from the direct action of cortisone on the mesoderm, with consequent poor absorption of food.

2. Skin: In the 12-day control chick embryo the feather papillae and medullae were densely cellular and had a well-developed blood supply. On cross section the medulla of the shaft was serrated and covered by several layers of epithelial cells. After the 14th day the surface cells had undergone keratinization. By the 18th day a thick layer of keratin had developed, overlying a thin, serrated epithelium with well-developed barbs (Fig. 1). The medulla gradually became more condensed, and by the 18th day had all but disappeared in the distal portion of the shaft. In the treated embryo, the epithelium became atrophic and underwent premature keratinization. Typical epithelial serrations and formation of barbs were not seen. The medulla was dilated and hypocellular, losing its serrations (Fig. 2). The blood vessels were fewer and less well developed than in the control embryos. The elaboration of the dermis was delayed. Whereas the dermis of the 18-day control embryo consisted of a well-defined layer of mature collagen fibers, in the 18-day treated embryo the dermis remained thin and was composed of delicate, loosely arranged collagen fibers. These changes were severest in the 18-day embryos injected with 3.75 mg. of cortisone acetate, but minimal changes were noted as early as the 12th day in the embryos which had been given only 0.5 mg.

3. Bones: The ossification of bones and formation of bone marrow were definitely delayed in the treated embryos. Ossification did take place, however, and proceeded in an orderly manner, starting in the skull.

4. Skeletal Muscle and Fat: In cortisone-treated embryos the development of the skeletal muscle and subcutaneous fat lagged several days behind that of the controls, depending upon the dose of cortisone and the length of the experiment.

5. Eyes: In addition to the disproportionately large eyes found in all the treated embryos, the 18-day embryos injected with 2.5 and 3.75 mg. of cortisone acetate had a retinal lesion. The basal layers of the retina were finely folded, acquiring a papillary architecture. The fact that this lesion was found only in the 18-day-old embryos suggests that it resulted from the increasing disproportion of the size of the eye socket

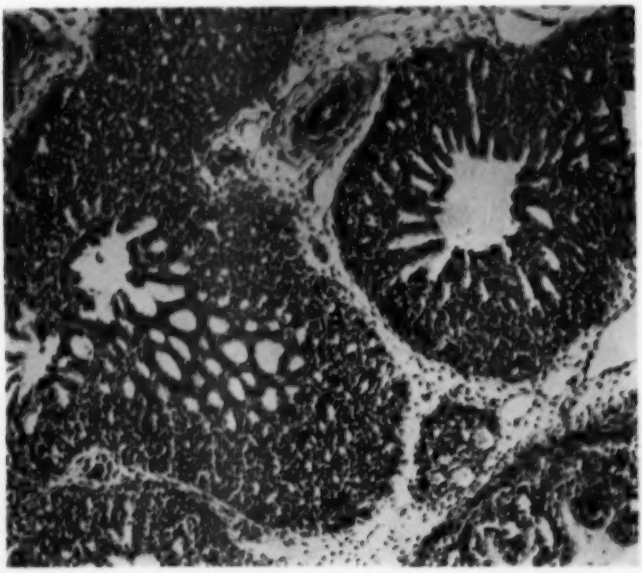


Fig. 3.—Normal lung, 18-day control. Hematoxylin and eosin; × 100.

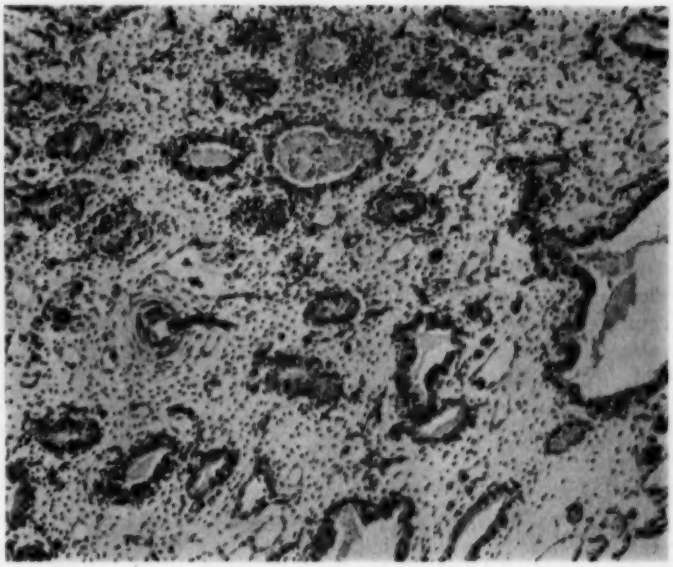


Fig. 4.—Lung, 18-day embryo treated with 3.75 mg. of cortisone acetate. Note lag of development of bronchi, hypoplasia of epithelium, and loose stroma. Hematoxylin and eosin; × 100.

and the size of the eyeball and, therefore, was a simple folding secondary to different rates of growth. In two embryos hemorrhages were noted in the anterior chamber of the eye, the cause and significance of which were not ascertained.

6. Lungs: One of the most striking effects of cortisone was the profound inhibition of the development of the bronchial tree. In the 12-day control embryos the bronchial tree consisted of simply branched major bronchi. By the 18th day respiratory bronchioles and air saccules had formed (Fig. 3). In the 18-day treated embryos (Fig. 4) the lungs resembled those of the 12- to 14-day controls (Fig. 5), differing in

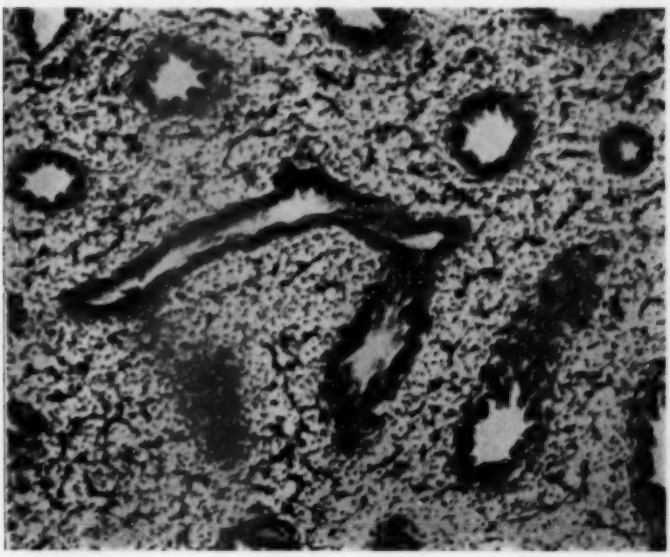


Fig. 5.—Normal lung, 14-day control embryo. Compare with Figure 4. Development of bronchial tree is at approximately the stage of the 18-day treated embryo. Hematoxylin and eosin; × 100.

that the vascular tree was better developed and markedly dilated, the mesoderm was loose rather than compact, and the bronchial epithelium was not as tall.

7. Pituitary Gland: Sections taken through the pituitary showed no histological changes that could be attributed to cortisone.

8. Thyroid: In the chick embryo the thyroid is fairly well developed by the 10th day. Some small follicles have formed, but there is little or no colloid. In the 18-day controls, the follicles were large, well developed, and filled with a pale-staining colloid. In the 18-day treated embryos the thyroids resembled those of the 10- to 12-day controls. The follicles were small, and there was little or no colloid present.

9. Liver: Hepatic damage appeared as early as the 12th day in the treated embryos, even in those given the smallest dose of cortisone (0.5 mg.). The cord cells appeared swollen, and the cytoplasm was finely vacuolated. In the embryos injected with 2.5 and 3.75 mg. of cortisone acetate, the cytoplasmic vacuoles were larger and often filled the cytoplasmic spaces. In focal areas cord cells with a hyalin-like cytoplasm were found, and some of the nuclei had undergone karyorrhexis. These areas were infiltrated with acute inflammatory cells (Fig. 6). The necrosis affected several lobules, and the distribution of necrosis varied from embryo to embryo. The amount of necrosis, however, correlated roughly with the dose of cortisone. In several of the 18-day treated embryos much of the necrotic debris had disappeared, suggesting that repair was taking place.

10. Kidneys: Subnuclear vacuoles in the convoluted tubules and swelling of the epithelial cells of Bowman's capsule were seen in the 12-day embryos injected with 0.5 mg. of cortisone acetate. In embryos given 2.5 and 3.75 mg. of cortisone acetate mononuclear cells with a vacuolated cytoplasm were found lying free within Bowman's capsule. Some of these cells contained material within their cytoplasm which stained with Sudan IV. The 14- and 18-day-old treated embryos showed diffuse and focal thickening of the basement membrane of the glomerular capillaries and of Bowman's capsule. Eosinophilic debris, which apparently represented disintegrated tubular cells, was found in the tubules (Figs. 7 and 8). In some of the 18-day-old treated embryos the

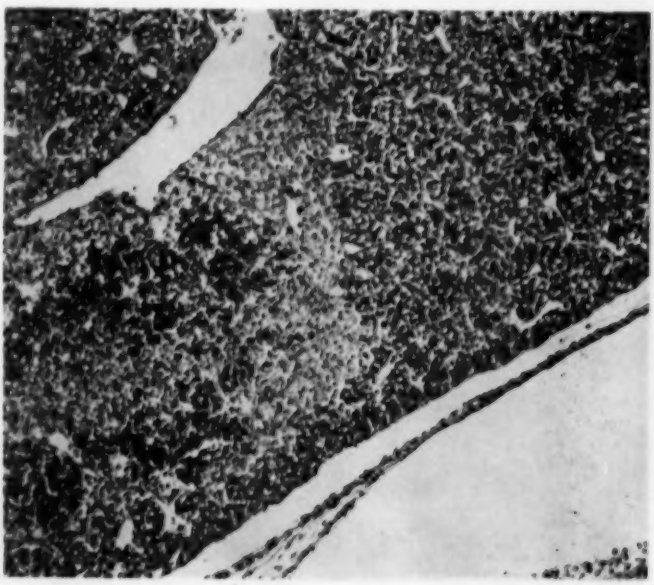


Fig. 6.—Liver, 18-day embryo treated with 3.75 mg. of cortisone acetate. Note diffuse cytoplasmic vacuolation of cord cells and large area of necrosis infiltrated with acute inflammatory cells. Hematoxylin and eosin; × 100.

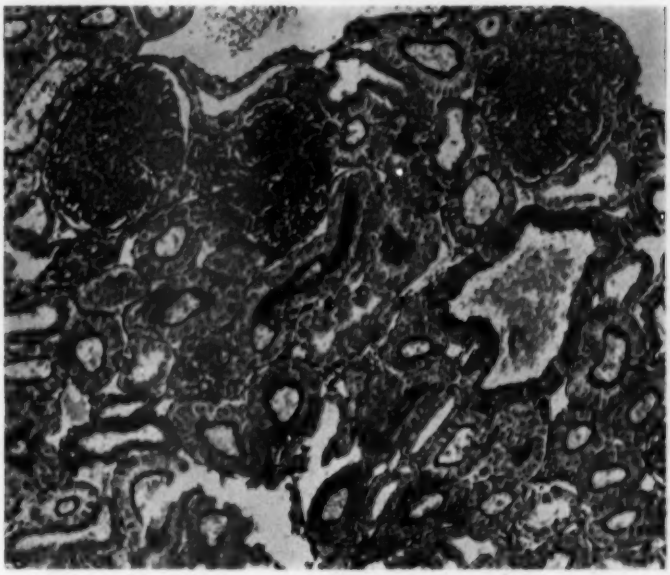


Fig. 7.—Kidney, 18-day control embryo. Hematoxylin and eosin; × 100.

tubular epithelium was intact, but the tubules were dilated and contained occasional large, basophilic concretions. In the embryos treated with smaller doses of cortisone the tubular changes remained minimal, and thickening of the basement membrane was not evident.

11. Gonads: No definite changes were noted in the ovaries. The testes, however, were retarded in development. In the 18-day control embryos some of the solid cords were beginning to form into tubules; no tubular formation had occurred in the testes of the treated embryos.

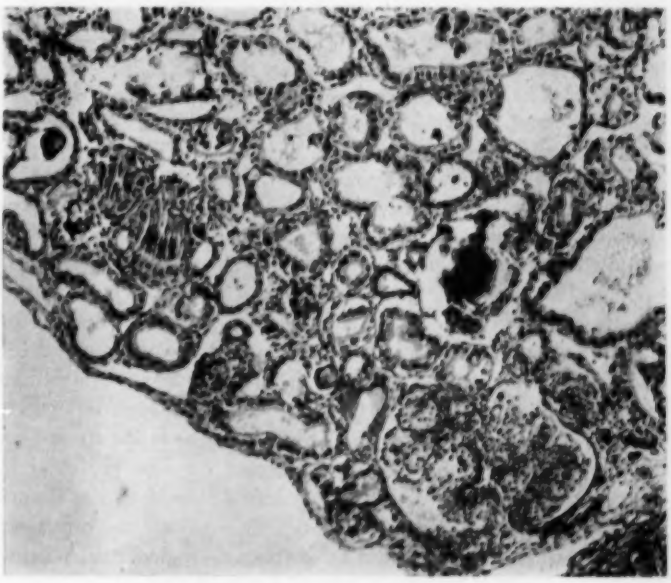


Fig. 8.—Kidney, 18-day embryo treated with 3.75 mg. of cortisone acetate. Note thickening of glomerular basement membrane, dilatation of tubules, and tubular concretions. Hematoxylin and eosin; × 100.

12. Heart: There was no evidence of any effect on the myocardium of the heart or on any of the peripheral vessels. Two of the 18-day embryos which had been given 3.75 mg. of cortisone, however, showed areas of necrosis in the epicardium, associated with an infiltration by acute inflammatory cells.

Comment

The effect of cortisone on various body tissues is not clearly understood. It has been particularly difficult to differentiate between the direct and the indirect effects of this hormone. Karnofsky [1] found that cortisone does not affect the growth rate of the chick embryo until after the 8th day of development, and that its effect is diminished after the 13th day. He postulated that cortisone interfered with some new substrate which normally appears during this period. The interval between the 8th and the 12th day is important in that many new functions develop during this time, some of which are known to be affected by cortisone. It is during this period that the mesonephros, having reached the peak of its development, gives way to the metanephros, which begins to excrete. The liver peptidase rises sharply.[3] The liver cells now have stainable glycogen and cholesterol.[4] The thyroid acini begin to elaborate colloid.[4] The pituitary growth hormone begins to exert its influence.[5] The effect of cortisone on the pituitary gland deserves special attention. Fugo [5] found that hypophysectomy had no appreciable effect on the growth and development of the chick embryo until after the 10th day of incubation. In this respect its action was similar to that of cortisone in our embryos. Furthermore, the 18-day hypophysectomized chick embryo had smaller than normal body proportions, a hypoplastic thyroid with deficient colloid formation, shorter and less well-developed down feathers, and deficient yolk sac development. Since all these deficiencies were noted in the treated embryos in our study, it could be inferred that these abnormalities are secondary manifestations of the direct action of cortisone on the pituitary. There is some experimental evidence to support this theory. Cortisone causes involution of the pituitary in the rat and mouse,[6] reduces the corticotropin content of the pituitary of dogs,[7] and reduces the blood thyrotropic hormone in young rats.[17] It may be that cortisone inhibits pituitary metabolism and thereby decreases the ability of the adenohypophysis to secrete one or more tropic hormones. In addition, there is evidence that exogenous cortisone not only disturbs the equilibrium between the corticotropin and the adrenal cortex but may have an antagonistic effect on the circulating thyroid and pituitary growth hormones. For example, the administration of cortisone slows the growth rate in young rats, but if thyroid is given the growth rate becomes normal.[8] Furthermore, some of the effects of cortisone in dogs can be neutralized by properly calculated doses of pituitary growth hormones.[9] The possibility that the thyroid, adrenal, and pituitary growth hormones are antagonistic bears investigation.

There are indications that cortisone has a direct effect on tissues, particularly the mesenchyme. Its influence on wound healing is well known. Cortisone is said to interfere in vitro with the glucose metabolism of adipose tissue [10] and to inhibit in vitro the growth of the chick embryo femur anlage.[11] In our experiment, the decrease in fat, the interference with the development of the dermis, as well as the feather papillae, and the delay in the development of bone and skeletal muscle could be attributed to the direct action of cortisone. Yet the indirect effects of the action of cortisone upon the pituitary and thyroid may also play a role. It was noted, for example, that in the hypophysectomized chick embryo the feathers were small and less well developed,[5] although this was apparently not as marked as in the treated embryos in our study.

The profound inhibition of the development of the bronchial tree may also result from the direct action of cortisone on the mesoderm. According to Locy and Larsell [12]

the mesoderm of the lung is of primary importance in the organization and development of the bronchial tree. If the mesoderm is inadequate, the bronchial tree does not develop.

The reason for the liver necrosis is not clear. Presumably cortisone interferes with the enzymatic activity of the cord cells. Fetal rats treated with cortisone show a decrease in the ribonucleic acid and alkaline phosphatase content of the liver parenchyma.[13] The liver glucose-6-phosphatase activity is increased, and there is an increase in liver glycogen and fat in young rats treated with cortisone.[14] Cavallero et al. found that cortisone reduced the ribonucleic acid and protein content of the whole chick embryo out of proportion to the body weight and suggested that cortisone interferes with protein synthesis.[15]

The renal lesions in embryos injected with cortisone were invariably associated with liver damage, but the relationship has not been elucidated. Rabbits treated with cortisone developed renal lesions consisting of deposition of lipid and an occlusion of the glomerular capillaries by a wax-like material.[16] A small amount of stainable lipid was found in the glomerular space in the treated chick embryos. The more prominent lesions in the chick embryo, however, were thickening of the basement membrane of the glomerular loop and of Bowman's capsule and deposition of basophilic concretions in the tubules. The latter were not birefringent and were not suggestive of amino acid crystals, sometimes seen associated with liver damage.

Summary

Administration of cortisone onto the chorioallantoic membrane of the chick embryo results in marked interference with growth and development that varies directly with the dosage.

Among the changes noted are (*a*) atrophy of the epithelium, premature keratinization, and lack of barb formation of the skin; (*b*) retardation of mesenchymal development, such as delay of ossification of bones and reduced growth of skeletal muscle and adipose tissue; (*c*) retarded development of the bronchial tree, including the epithelium, and (*d*) necrosis of liver cord cells, and glomerular and tubular degeneration in the kidney.

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Saturated Versus Unsaturated Fats in Experimental Arteriosclerosis

Comparison of the Effect of Various Types of Fatty Meals on Thromboembolic-Induced Pulmonary Arteriosclerosis in Rabbits

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Considerable clinical, pathologic, and experimental evidence [1-7] has accumulated indicating that arteriosclerosis in the small pulmonary arteries of man can result from repeated showers of thromboemboli. In this type of arteriosclerosis the initiating event is apparently the lodging of a small thromboembolus in a pulmonary vessel, and this event is followed by fibrosis and fat deposition.

We have performed a series of experiments designed to test the effect of various factors on thromboembolic-induced pulmonary arteriosclerosis in rabbits.[8,9] We believe that basic data obtained from these experiments should be useful not only in investigating the special types of pulmonary arteriosclerosis known to result from thromboembolism but also in investigating factors that influence lipoidosis, fibrosis, and thrombosis in any form of arteriosclerosis.

In previously reported experiments [9] we have shown that weekly feedings of butter or oleomargarine markedly increase the yield of thromboembolic-induced pulmonary arterial lesions in rabbits. Butter and oleomargarine had essentially the same effect, in spite of the fact that the type of oleomargarine used was entirely of vegetable origin.

Recent studies by Friskey, Michaels, and Kinsell [10] and others [11,12] suggest that the degree of saturation of a fat rather than its source may be the important factor in determining its biological effect. Since butter and oleomargarine are both highly saturated, we decided to test the effect of corn oil, a relatively unsaturated fat, on thromboembolic-induced pulmonary arteriosclerosis in the rabbit and to compare the results with those obtained with the saturated fats. The purpose of this report is to present the results of our study.

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From the Department of Pathology, Washington University School of Medicine.

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Material and Methods

Young, adult New Zealand White rabbits of both sexes were used as experimental animals. They were kept in air-conditioned rooms, fed 100 gm. of Purina Rabbit Pellets daily, and offered water ad libitum.

The methods of stomach feeding and clot injection have been described in detail in a previous report,[9] and only a summary will be given here. The experiments involving corn oil were carried out as nearly as possible in exactly the same fashion as the previous experiments in which butter and oleomargarine were used. However, in order to ensure against unconscious changes, additional groups were established receiving butter or water containing an amount of sugar sufficient to supply the same amount of calories present in the fat fed to the other groups.

As before,[9] blood was obtained by cardiac puncture from other healthy rabbits and partially defibrinated by beating. The resulting clot was then placed in a Waring Blendor and mixed with saline. This clot-saline mixture was injected into the ear vein of each rabbit through an 18-gauge needle. Each rabbit received clot from 10 ml. of blood once weekly for six injections (total, 60 ml.). At the time of intravenous injection a rubber tube was passed transorally into the stomach of each rabbit and 25 ml. of warm corn oil, melted butter, or a warm saturated sucrose solution was

given. Thus three groups were established. Fifteen rabbits received corn oil; 10 received butter, and 10 received an isocaloric amount of sucrose and water. Otherwise, the three groups were treated in the same manner, and every effort was made to conduct the experiment in the same fashion as before.[9]

All the rabbits were killed with intravenous formalin one week after the last injection, and autopsies were performed. One block of tissue was taken from each upper and each lower lobe of the right and left lungs for paraffin sections, and corresponding blocks were taken for frozen sections. Sections from all paraffin blocks were stained with aldehyde fuchsin-Van Gieson-iron hematoxylin (for elastic and connective tissues), and frozen sections were stained with oil red O (for fat). Lesions found in the small pulmonary arteries were classified as either thrombi or fibrous intimal thickening and were graded according to a system previously described in detail.[8,9] In brief, the rabbits were divided into two groups on the basis of the extent and severity of fibrous intimal lesions in the pulmonary arteries: 1. Those with three or more fibrous intimal lesions that were as thick as or thicker than the adjacent media were classified as having moderate or advanced pulmonary arteriosclerosis. 2. Those with less than three lesions that were as thick as or thicker than the adjacent media were classified as having slight or no pulmonary arteriosclerosis. The microsections were examined and graded objectively without the examiner knowing what the rabbits had received. The four sections from each rabbit stained for elastic and connective tissue were considered as a single unit for grading purposes.

Results

No gross lesions were observed in the pulmonary arteries. Microscopic lesions were confined to the small pulmonary arteries and were indistinguishable from lesions that have been described previously in rabbits receiving intravenous blood clot with or without fatty meals.[1,2,9] The lesions were qualitatively similar in all groups and were of two general types. One type was a clearly recognizable thrombus, often with multiple channels, and the other consisted of fibrous intimal thickening characteristic of pulmonary arteriosclerosis. There was no sharp dividing line between the two types, although most lesions could be classified readily as one or the other. Fat was demonstrated in only occasional lesions. However, some fat was demonstrated in the lesions of rabbits in all groups. The quantity of fat was not appreciably greater in the rabbits that had received fatty meals than in those that had not received fatty meals.

TABLE 1.—*Number and Percentage of Rabbits in the Various Groups Reported Herein with "Moderate" or "Severe" Fibrous Intimal Thickening of Their Small Pulmonary Arteries*

Groups*	No. in Experiment	No. with Moderate or Severe Lesions	Percentage with Moderate or Severe Lesions
Clot-butter	10	7	70
Clot-corn oil	15	6	40
Clot-sugar solution	10	4	40

* Clot given intravenously and fat or sugar solution introduced into stomach, as described in the text

The results of the grading of the extent and severity of the fibrous intimal lesions are summarized in the Tables. In the current experiment (Table 1) 70% of the rabbits that received butter were classified as having "moderate" or "advanced" pulmonary arteriosclerosis. The corresponding percentage for those that received corn oil is 40, and the same percentage (40) was obtained in those receiving sugar and water. In Table 2 we have summarized all of our results to date with fatty meals and thromboembolic-induced pulmonary arteriosclerosis by combining the data from previous experiments with those obtained in the current experi-

TABLE 2.—*Summary of Number and Percentage of Rabbits in Various Corresponding Groups Reported in This and in Previous Experiments with "Moderate" or "Severe" Fibrous Intimal Thickening of Their Small Pulmonary Arteries*

Groups*	Total No. in Group	No. with Moderate or Severe Lesions	Percentage with Moderate or Severe Lesions
Clot-butter	25	20	80
Clot-oleomargarine	12	9	75
Clot-corn oil	15	6	40
Clot-water	15	6	40
Clot-sugar solution	10	4	40
Saline*-butter	4	0	0
Saline*-oleomargarine	4	0	0
Untreated	25	0	0

* Clot suspension or saline given intravenously in each instance and fat or water or a sugar solution introduced into the stomach as described in the text.

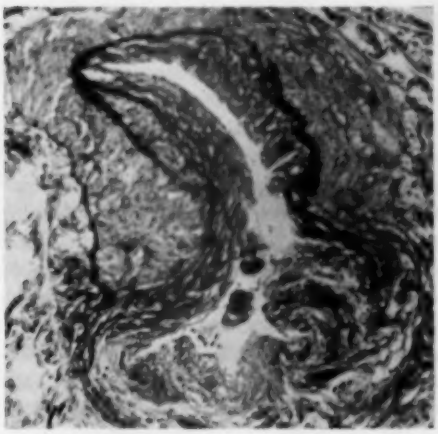


Fig. 1.—A fibrous intimal lesion in a small pulmonary artery of a rabbit that received intravenous injections of blood clot and supplementary feedings of butter. Aldehyde fuchsin-Van Gieson stain; × 100.

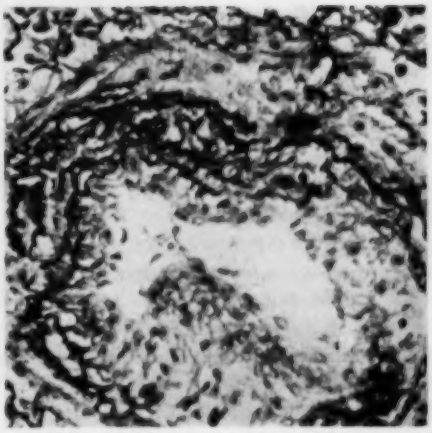
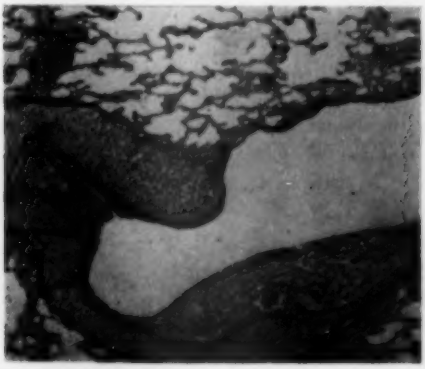


Fig. 2.—A fibrous intimal lesion in a small pulmonary artery of a rabbit that received intravenous injections of blood clot and supplementary feedings of corn oil. Aldehyde fuchsin-Van Gieson stain; × 250.

ment. Butter and oleomargarine have a similar marked effect on the incidence of thromboembolic-induced pulmonary arterial lesions, but corn oil has no more effect than plain water or water containing sucrose. The difference between the effect of butter (80% with "moderate" or "advanced" pulmonary arteriosclerosis) and the effect of corn oil (40% with "moderate" or "advanced" pulmonary arteriosclerosis) is statistically significant ($P = 0.05$).

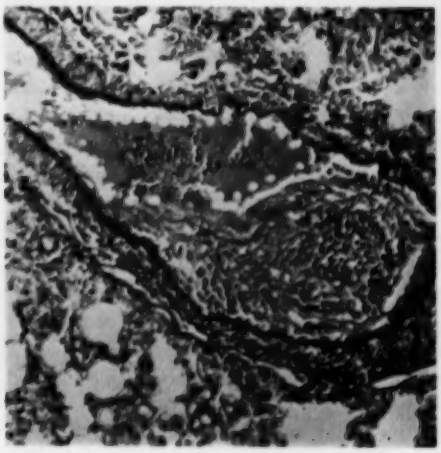
Fig. 3.—A fibrous intimal lesion in a small pulmonary artery of a rabbit that received intravenous injections of blood clot and supplementary feedings of sugar. Aldehyde fuchsin-Van Gieson stain; × 60.



Comment

It has been demonstrated in humans by others [10,12] that diets containing saturated fats increase the level of cholesterol in the blood, whereas unsaturated fats may actually lower the cholesterol level. Our experiments indicate that butter (a highly saturated fat) and corn oil (an unsaturated fat) may differ in their biological effects in still an-

Fig. 4.—A fibrous lesion in a small pulmonary artery of a rabbit that received intravenous blood clot and supplementary feedings of sugar. This lesion may represent an intermediate stage between an occluding thrombus and an eccentric plaque. Aldehyde fuchsin-Van Gieson stain; × 120.



other way. Feedings of butter markedly increased the yield of pulmonary arterial lesions associated with thromboembolism, whereas corn oil had no demonstrable effect. The fact that oleomargarine of vegetable origin had the same effect as butter suggests that the difference is not simply a matter of origin (vegetable or animal). We have not proved that the difference in effect of butter and corn oil is simply a matter of the degree of saturation, but this appears to us to be the most likely explanation. However, another possibility is that the difference in effect is related in some way to the essential fatty acid content of the various fats, and there are undoubtedly still other possibilities.

The mechanism by which fatty meals of butter and oleomargarine increase the yield of thromboembolic-induced pulmonary arterial lesions is not clear. We have demonstrated previously [8] that the injection of nonthrombotic occlusive bodies, such as small plastic spheres, into small pulmonary arteries does not result in the production of arteriosclerotic lesions (at least not in an experiment of similar duration to that of the experiments in which lesions are produced with clot). We have demonstrated in previous studies of pulmonary arteries in humans and rabbits what appear to be transition forms between clearly recognizable thrombi and arteriosclerotic plaques.[6,9] Hence we have concluded that the arteriosclerotic lesions produced in the experiment reported herein are a direct result of the organization of thrombi. This conclusion has led us to hypothesize that the fatty meals of butter and oleomargarine must act by inhibiting the forces in the blood stream that result in the disintegration of thrombi (such as fibrinolysin) or by increasing the fibroblastic reaction of the vessel wall.

Whatever the mechanism of the biological effect may be, the most important observation in this experiment is that butter and oleomargarine (saturated fats) have a marked effect on thromboembolic-induced lesions in the rabbit, whereas corn oil (a relatively unsaturated fat) has no effect.

Thus, from previous experiments performed by others and from our experiments, we know that certain highly saturated fats may cause a rise in the serum cholesterol level in man [10,12] and may increase the yield of thromboembolic-induced pulmonary arterial lesions in rabbits. It should be emphasized that these data are insufficient to justify specific conclusions regarding the etiology and pathogenesis of systemic atherosclerosis and warrant only a certain amount of thoughtful speculation. If atherosclerosis is caused by a high cholesterol level in the blood which results in the deposition of fats in the intima, and thrombosis is only a late complication, then appropriate fatty meals might affect both the basic disease and the complication but at different times. If systemic atherosclerosis is basically a mechanistic affair resulting from the deposition of thrombi on the vessel wall or from hemorrhage within, then appropriate fatty meals might affect the disease in the same fashion throughout its course. In any event, our experiments suggest a pathway along which several apparently widely different avenues of thought regarding the etiology and pathogenesis of systemic atherosclerosis and its complications might converge.

Summary

Recently, considerable evidence has been presented indicating that arteriosclerosis of the small pulmonary arteries of man can result from repeated showers of thromboemboli.

Several investigators have produced pulmonary arteriosclerosis in rabbits by the repeated intravenous injections of blood clots. We have used this method of producing pulmonary arteriosclerosis in rabbits as a tool to test some of the biological properties of various types of fatty meals.

In previous experiments we have demonstrated that occasional fatty meals of butter or oleomargarine markedly increase the yield of arteriosclerotic lesions resulting from thromboembolism in the rabbit. In the experiment reported herein we have demon-

strated that occasional fatty meals of corn oil (in quantities similar to the meals of butter and oleomargarine used previously) have no effect on thromboembolic-induced pulmonary arteriosclerosis in the rabbit.

Although we have emphasized the possible role of the degree of saturation of various fats in the report, the possibility has not been excluded that other factors, such as the essential fatty acid content, may be equally or more important.

At least in one specific situation, butter and oleomargarine (highly saturated fats) have a profound biological effect, whereas corn oil (a relatively unsaturated fat) has no demonstrable biological effect.

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Congenital Segmental Stenosis of the Umbilical Vein

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Intrinsic defects of the umbilical vessels that may account for intrauterine death have received little study, as judged by a review of the literature on the subject. The most important anomalies of the umbilical cord recorded in the literature are knots, entanglements, or wrappings due to excessive length and procidencia. The anomaly of the umbilical vein reported by Putschar [1] and Shryock et al.[2] in two newborn infants represented an anatomical anomaly of the usual pathway of umbilical vein, ductus venosus, and inferior vena cava, without appreciable interference with the dynamics of the fetal circulation and physiology. In both cases the umbilical vein by-passed the liver, pierced the diaphragm, and opened directly into the right auricle of the heart, lateral and somewhat anterior to the ostium of the inferior vena cava. In both cases multiple associated congenital abnormalities were found which were incompatible with extrauterine life. The case presented below is that of a stillbirth in which a severe degree of stenosis of the umbilical vein represented the only congenital anomaly and may, in our opinion, have contributed to the production of intrauterine anoxia.

Report of a Case

A 34-year-old white woman, tertigravida, secundipara, was admitted to Jewish Memorial Hospital on March 28, 1956, in labor, her first pains having started one hour and a half before entry. She had had two previous pregnancies, which had resulted in two uneventful, spontaneous deliveries, one in 1949 and the other in 1952. Both babies were full term, the first one weighing at birth 5 lb. 10 oz. (2550 gm.) and the second 7 lb. 1 oz. (3203 gm.). Serology was negative. The mother's blood type was O Rh-positive. There was no laboratory evidence of Rh or ABO sensitization. The past history was found to be quite irrelevant.

The patient's present 40-week pregnancy had progressed satisfactorily to the day prior to admission to the hospital, at which time a period of marked fetal activity was followed by quiet. Six hours prior to admission fetal heart tones were not audible. On admission, the labor pains lasted 30 seconds and occurred at intervals of 2 minutes; the membranes were intact, and there was no bleeding. The cervix was two fingers dilated; the presentation was cephalic and at Station two. The blood pressure was 130/80; the pulse, respiration, and temperature were within normal limits. Fetal heart tones were still not heard. Labor lasted four hours and ended in the unaided delivery of a full-term stillborn female infant in LOA position. There was a loop of the umbilical cord around the neck, which had to be severed between two clamps. The cord was of normal length, measuring 50 cm. Just above the umbilicus it showed a fusiform, dark-red swelling, which was interpreted as a hematoma or a hemangioma. There was no sign of compression of the soft tissues of the neck by the cord. The placenta and membranes were intact and normal in appearance. They were expelled shortly after delivery.

Autopsy Findings

The necropsy was started 12 hours after delivery.

The body was that of a full-term well-developed white female infant, weighing 3300 gm. and measuring 30 cm. in crown-rump and 50 cm. in crown-heel length. The skin of the face, neck, trunk, and extremities was intact and of natural, reddish-pink color. Externally the only abnormality was a dark-red, fusiform swelling of the umbilical cord, measuring 3×1.5 cm., the proximal part of which was in the immediate vicinity of the skin of the umbilicus. Entering the swelling was a tuft of dilated and tortuous vascular channels, engorged with blood. The tissue of the cord at the demarcation line of the tumefaction presented a whitish, opaque appearance and felt somewhat firm. At that point further dissection disclosed a marked narrowing of the umbilical vein, the lumen of the vessel barely admitting a 20-gauge needle. Longi-

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Associate Pathologist (Dr. Alerte). Resident in Pathology (Dr. Payan).



Fig. 1.—Segment of umbilical cord showing umbilical vein (*UV*), umbilical arteries (*UA*), and dark-red swelling situated between the umbilical skin and the stenosis, the length of which is represented by the portion of the cord outlined by the solid parallel lines.

tudinal incision of the umbilical vein revealed the defect to involve a 0.5 cm. segment of the vessel. The lumen of the vein at that point was deflected laterally, and the intima was thickened. The Wharton's jelly around the narrowing was condensed, whitish in color, and of fibrous texture. The umbilical arteries were patent throughout, but their diameter was somewhat smaller than usual. Proximal and distal to the narrowing there was no appreciable change in the caliber or appearance of the umbilical vein. The dark-red, fusiform swelling previously mentioned was situated between the stenosis and the umbilical skin. It was formed by a spongy-appearing tissue supporting a network of ectatic vascular channels, some of which bulged outwardly to form the superficial tuft mentioned above. The others coursed for short distances in the inner portion of the dark-red, spongy tissue of the cord in the direction of the umbilicus. Deep or superficial, those vascular channels ended all as blind pouches, and their origin could be easily traced to the trunk of the umbilical vein. The largest one measured 0.8 cm. in its greatest diameter. Examination of the viscera, except for turgid congestion, hemorrhages, and atelectasis of the lungs, revealed no noteworthy pathological changes. The ductus venosus and inferior vena cava were patent and appeared quite natural. The cardiovascular system was well developed and showed no changes. The falx cerebri and tentorium cerebelli were intact; the dural sinuses were distended with blood. The brain was of natural appearance except for marked passive hyperemia of the leptomeninges.

Microscopic Findings

Section of the stenotic portion of the cord showed the intima of the umbilical vein to be represented by a strip of hyalinized connective tissue bearing a few spindle-shaped, pyknotic nuclei. The media exhibited considerable muscular atrophy. The adventitia merged inconspicuously with the Wharton's jelly, which appeared shrunken, fibrillated, and hyalinized. No inflammatory infiltration was discernible. The umbilical

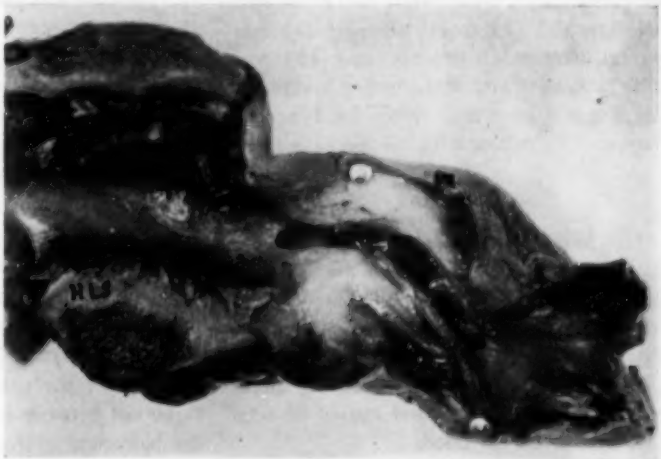


Fig. 2.—Umbilical vein longitudinally incised. Note the deflection of the vein lumen, the condensation of Wharton's jelly at the stenosis site (distance between the two dots on the photograph), the cross section of one of the umbilical arteries (*UA*), and the spongy appearance of the adjacent tissue of the umbilical cord (*HLS*). Reduced 69% of mag. × 9.

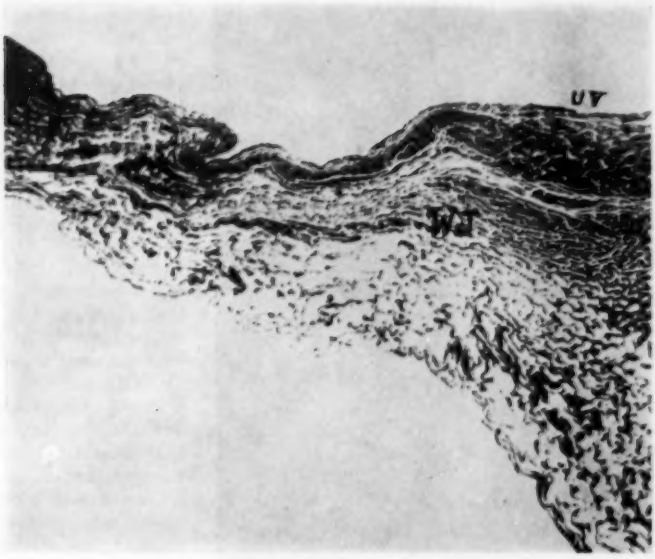


Fig. 3.—Low-power photomicrograph of umbilical cord. The thin end of the section is from the region of luminal narrowing. Note the decrease in thickness of the wall of the umbilical vein (*UV*) from right to left and observe, at the same time, the progressive shrinkage of the underlying Wharton's jelly (*WJ*). Reduced to 83% of mag. × 25.

arteries contained no thrombi. One of them showed a moderate degree of medial atrophy. Section of the umbilical cord taken at the level of the dark-red tumefaction grossly described showed disruption of the wall of the umbilical vein and cord by ectatic capillary channels engorged with blood. Small and large hemorrhages resulting from the rupture of the wall of some of those channels were seen here and there. Sections of the viscera showed marked acute passive congestion. The lungs showed, in addition, numerous focal hemorrhages and atelectasis.

Diagnosis: (1) umbilical vein: (*a*) congenital, segmental stenosis, and (*b*) vascular anomaly (of undetermined nature); (2) lungs: atelectasis with focal hemorrhages; (3) intrauterine anoxia.

Comment

The immediate cause of death in some instances of intrauterine anoxia is very difficult to establish. Some form of compression of the umbilical cord often accounts for fetal circulatory embarrassment that occurs immediately before or during labor, when maternal or placental causes of fetal distress can be excluded.

In the present case, the clinical and pathological evidence suggests the possible interplay of two factors which occasioned a reduction of the oxygen supply late in the course of the pregnancy. These two factors were (1) the stenosis of the umbilical vein and (2) the wrapping of the cord around the neck of the baby. It is well known that the umbilical cord may be wrapped several times around the neck of a fetus without producing evidence of fetal distress. However, if the portion of the cord between the placenta and the point of entanglement is short, this segment may be placed under excessive tension, and the lumen of the blood vessels may be greatly reduced as the fetus moves down in the birth canal during labor. Most fetuses dying in utero of such complication show at birth evidence of recent strangulation, such as cyanosis of the face and head and some compression of the soft tissues of the neck. Moreover, the segment of the cord between the entanglement and the placenta is swollen, owing to stasis and edema. In this instance, though the loop of the cord around the neck had to be cut between two clamps, the body of the baby was uniformly pink, and the two

portions of the cord were of equal thickness. Had the umbilical vein been of normal caliber throughout, a sufficient amount of blood seemed capable of reaching the fetal circulation to keep the fetus alive. Since, at the point of the stenosis, the umbilical vein barely admitted a 20-gauge needle, we can infer that any further reduction in the volume of blood flow may well have had a deleterious effect on the circulation to the fetus.

The ectatic vascular channels encountered between the stenosis and the umbilicus are interesting findings, but their significance is not very clear to us. We are not able to decide whether they represent developing collateral channels or fortuitous coincidental hemangiomatous aberrations.

In the region of narrowing no amniotic adhesions were found grossly, and on microscopic examination there was no cellular inflammatory infiltration. A careful inquiry elicited no history of rubella or other viral infections in the mother. No adequate explanation is at hand to account for this rare lesion.

To the embryologist, congenital segmental stenosis of the umbilical vein offers an intriguing, and possibly bewildering, abnormality which cannot be explained on the basis of developmental changes. To the pathologist and the clinician this anomaly may provide a rare mechanism for some of the too many baffling cases of intrauterine anoxia.

Summary

A case of congenital segmental stenosis of the umbilical vein is described, associated with an anomalous vascular tissue in the adjacent portion of the umbilical cord. No similar instance could be found in the literature. No adequate explanation can be offered as a mechanism for its occurrence.

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Production of an Area of Homogeneous Myocardial Infarction in the Dog

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With the Technical Assistance of Zigmund E. Zudyk, B.S.

Introduction

Ligation of a major coronary artery in the dog usually results in the development of a large myocardial infarct, which on gross inspection appears uniformly necrotic. However, microscopic examination shows that the appearance of uniformity is deceptive, for in many places islands of normal tissue are spared and the necrosis is patchy. The explanation for this finding is that the dog possesses an extensive collateral coronary circulation which apparently is sufficient to prevent the death of some of the myocardial fibers in the area of the gross infarct.[1,2] Thus the ordinary myocardial infarct produced by ligation of a large coronary artery is not a homogeneous one, but, instead, is a heterogeneous mixture of unknown proportions of necrotic and living myocardial fibers which varies from animal to animal. Such a heterogeneous infarct is poorly suited for quantitative biochemical and morphological studies of the changes occurring during ischemia because the presence of normal tissue will tend to dilute the alterations in the injured cells.

For some time now we have investigated ways of producing an experimental infarct that would contain a minimum of normal myocardium and so reduce this source of error as much as possible. The first attempt involved ligation of the apical branch of the anterior descending ramus of the left coronary artery. This procedure resulted in the development of an infarct in the inner half of the anterior apical portion of the left ventricle. Both the location and the volume of the gross infarct were quite constant in dogs with a normal vascular pattern,[3,4] and the operation had almost no mortality rate. Such a lesion is obviously well suited for the experimental investigation of subendocardial infarction or for comparative studies of changes in the size of gross infarcts. However, observation soon disclosed that the infarct was not homogeneous and that the collateral circulation from both the right and the left coronary artery was sufficient to permit survival of some myocardial fibers. Efforts were therefore made to interrupt this collateral circulation, but since the operation proved to be technically impractical, another way of producing a homogeneous infarct was sought.

A clue to the solution of the difficulty was found in the earlier work of Burchell,[5] who, in a study of the adjustment of the coronary circulation in dogs after experimental occlusion of various major coronary arteries, incidentally observed complete fibrosis of the posterior papillary muscle following ligation of the circumflex branch of the left coronary artery. It occurred to us that if the infarct in the posterior papillary muscle were indeed uniformly necrotic it might be ideal for use in investigations of the biochemical and morphological lesions developing during the first few hours of infarction. In addition, it would have the advantage of a predictable location. The purpose of this paper is to give an account of the method of producing such a standard infarct and to describe its characteristics.

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From the Department of Pathology, Northwestern University Medical School.

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Experimental Procedure

Twenty-three mongrel dogs of both sexes were used for the experiments. The animals were kept in an air-conditioned room where the temperature was 70 C, fed Borden's Dog Meal, and allowed to drink as much water as they wanted. Ten to fifteen minutes before operation they were given an intramuscular injection of 50 mg. of procainamide hydrochloride U. S. P. (Pronestyl) per kilogram of body weight and then anesthetized with an intravenous injection of 1 ml. of pentobarbital (Nembutal) sodium (60 mg. of the drug per cubic centimeter) for each 5 lb. (2.3 kg.) of body weight. During the operation oxygen was administered intratracheally under positive pressure. The pericardium was opened aseptically through an incision in the left fifth intercostal space, and the circumflex branch of the left coronary artery was ligated with silk. The chest was closed with silk sutures, leaving the pericardium open, and the residual air in the pleural cavity was aspirated.

In the first 7 dogs both the circumflex artery and the vein were included in the ligature, but in the other 16 dogs only the artery was ligated (Table 1). This modification in technique made no difference in the results. Electrocardiograms were taken on all animals before, during, and after ligation of the coronary artery, using a Sanborn Model 51 direct-writing electrocardiograph. All dogs were allowed to survive for 24 hours or longer so that the characteristic morphological features of infarction could develop.

When the animals were killed, the heart was removed and immediately placed in a large volume of 10% neutral formalin and fixed for 24 hours. The coronary arteries were dissected from the myocardium and the site and effectiveness of the ligature determined. A note was made of the number of small ventricular branches that lay distal to the ligature, since it has been shown that the mortality rate of the operation correlates well with this finding.[6] Our results confirm this observation.

TABLE 1.—*Results Obtained by Ligating Left Circumflex Coronary Artery Near its Origin*

Animal No.	Sex	Weight, Lb.	Vessels Tied*	Survival Time	Homogeneous Infarct Posterior Papillary Muscle
69	F	...	a	24 hr.	+
82	F	24	a	25 hr.	+
54	F	12	a & v	25 hr.	+
79	M	21	a	25 hr.	+
86	M	18	a	25 hr.	+
71	F	19.5	a	49 hr.	+
57	F	30	a & v	47 hr.	+
59	...	12	a & v	71 hr.	+
156	F	42	a	71 hr.	+
68	F	18.5	a	73 hr.	+
60	F	11	a & v	10 days	+
61	F	11	a & v	106 days	+

*a=artery; a & v=artery and vein.

Samples of the infarct were taken from the posterior papillary muscle and the adjacent wall of the left ventricle. They were embedded in paraffin, and sections were cut and stained with hematoxylin and eosin, Heidenhain's connective tissue stain,[7] and the periodic acid-Schiff method.[8] Samples of control (normal) myocardium were taken from the anterior superior portion of the interventricular septum and the anterior wall of the left ventricle between the anterior papillary muscle and the interventricular septum, since these places were never involved in the infarcts. The control samples were treated exactly the same as the experimental tissues.

In order to estimate the extent of the circulation still existing after ligation of the left circumflex coronary artery, the distribution of blood within the infarct was studied by the use of isotopes. Fifteen minutes before the animal was killed serum albumin labeled with I^{131} was injected intravenously into two dogs. Samples of blood obtained from the left auricle were centrifuged and the activity of the plasma measured in a well-type Geiger-Müller counter. Duplicate samples of tissue were quickly taken from the normal left ventricle and the infarct in the posterior papillary muscle, blotted on filter paper to remove excess fluid, and placed in tarred plastic tubes. After weighing the tissue, its activity in counts per minute was determined. Since the activity of both the tissue and the plasma in counts per minute was known, the quantity of plasma present in 1 gm. of tissue could be calculated from the formula

$$\frac{\text{Counts/min. in tissue}}{\text{Weight of tissue, gm.}} \div \frac{\text{Counts/min/cc. plasma}}{} = \text{cc. plasma/gm. of tissue}$$

Because it was thought that some of the labeled protein might possibly diffuse through the walls of ischemic vessels in the margins of the infarct, the experiment was repeated using erythrocytes labeled with Cr^{51}, after the method of Sterling and Gray,[9] on the assumption that they would be less likely than plasma to leave the intravascular space. Fifteen cubic centimeters of autogenous erythrocytes labeled with 200μc of sodium chromate ($Na_2Cr^{51}O_4$) were injected into Dog 82, and the activities of a sample of heparinized blood and of tissue from the locations described above were measured and the results reported in cubic centimeters of blood per gram of tissue (Table 2).

Results

A few seconds after the left circumflex coronary artery was tied near the aorta, the lateral and posterior surfaces of the left

TABLE 2.—*Estimated Blood Content of Normal and Infarcted Tissue Fifteen Minutes After Injection of I^{131}-Labeled Serum Albumin or Cr^{51}-Labeled Erythrocytes*

Animal	Hours After Ligation	Substance Injected	Infarct in Post. Papillary Muscle	Infarct in L.V. Beneath Post. Papillary Muscle	Control Normal Left Ventricle
			Cc. Plasma/Gm. Heart Muscle		
69	24	80 μc I^{131} labeled serum albumin	0.018	0.021	0.04
71	48.5	120 μc I^{131} labeled serum albumin	0.0036	No sample taken	0.083
			Cc. Blood/Gm. Heart Muscle		
82	24	10 cc. of blood labeled with 200 μc of $Na_2Cr^{51}O_4$	0.0138	0.021	0.043

ventricle became markedly cyanotic and then the electrocardiogram showed marked elevation of the S-T segments in Leads II, III, and aV_f (Fig. 1). Several seconds later the heart dilated. During the 1st hour seven dogs died of the effects of ventricular fibrillation, and four other animals died of unknown causes 2 to 16 hours after the ligation. All of these animals were discarded because none of them had lived long enough to develop unmistakable evidences of necrosis. The five animals killed at the end of 24 hours all had a large light-tan infarct in the area that had turned cyanotic at the time of operation. Microscopic examination showed that the infarct involved the entire thickness of the ventricle, including the posterior papillary muscle. The portion of the infarct in the wall of the left ventricle usually showed patchy necrosis of the outer, or subepicardial, two-thirds and fairly uniform necrosis of the inner, or subendocardial, one-third. The posterior papillary muscle was always entirely necrotic except for a very thin layer of normal myocardium, 2 to 5 fibers in thickness, just beneath the endocardium (Figs. 2A and 2B). Two dogs killed at about 48 hours, as well as three killed after 70-72 hours showed similar findings. In animals that survived for a sufficient length of time the fresh infarct was gradually replaced by scar tissue. For

Fig. 1.—Typical electrocardiographic changes which follow ligation of the circumflex branch of the left coronary artery in dogs with a normal vascular pattern (Dog 156). Note the early S-T segment elevation in Leads II, III, and aV_f. The five-hour record shows the appearance of Q waves and inversion of the T waves in these leads.

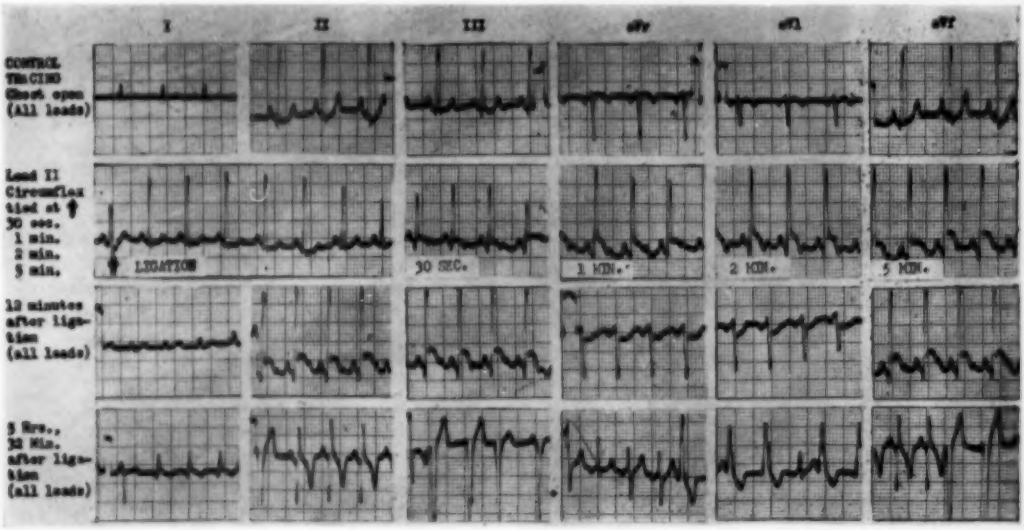




Fig. 2A (Dog 103).—Section of the full thickness of the left ventricle to illustrate the extent of the infarct in the posterior papillary muscle and lateral wall. The section was taken 2 hours 10 minutes after ligation of the left circumflex coronary artery and stained for glycogen. In the posterior papillary muscle there is no stainable glycogen, indicating that uniform injury has occurred, whereas in the underlying myocardium of the left ventricle the loss of glycogen is patchy. Periodic acid-Schiff method; ×3.

example, in Dog 61, which survived 106 days, the posterior papillary muscle had contracted to about 15% of its normal volume, and the only normal tissue remaining was a tiny island of intact myocardial fibers at the base where the muscle attached to the ventricular wall. Identical results were obtained regardless of whether the artery alone or both the artery and the vein were tied. Table 1 gives the essential data for individual animals.

Dissection of the coronary arteries revealed that the ligatures in all the dogs had completely occluded the left circumflex branch. On the average, the ligature was placed 11 mm. (range 1-21 mm.) from the origin of the artery in the aorta, and two of the small ventricular branches (range 1-4) were left in the portion of the artery that was proximal to the tie.[2] The mortality at one hour was 31%, and by the end of 24 hours had increased to about 45%.

The results of the experiment shown in Table 2 indicate that, even after complete obstruction of the left circumflex coronary artery by ligation, isotopically labeled plasma and red blood cells entered the infarct. However, the amount of tracer substance in the infarct was much less than in control tissues from normal left ventricle. In the posterior

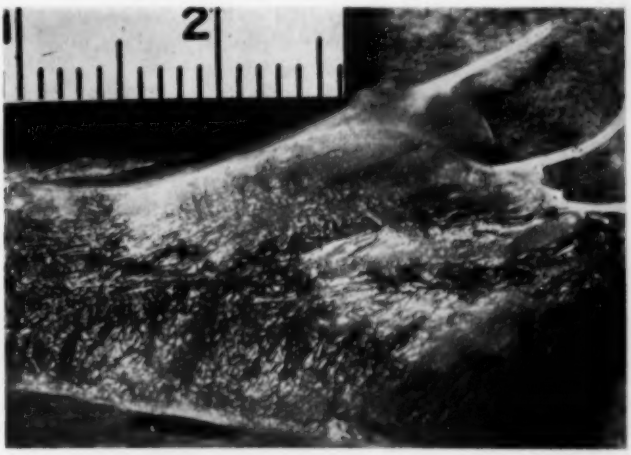


Fig. 2B.—Gross appearance of the same infarct as that in Figure 2A; ×3.

papillary muscle, where the infarct was uniform, the amount of tracer was always less than in the posterolateral portion of the wall of the left ventricle, where the infarct was not uniform.

Comment

The results of these experiments show that soon after the left circumflex coronary artery is ligated near its origin a large infarct develops in the posterolateral portion of the wall of the left ventricle. The posterior papillary muscle is always involved in the infarct and is remarkable because few or no living cells survive and the necrosis is thus very uniform. For these two reasons—the predictable location and the uniform necrosis—the posterior papillary muscle is well suited for use in quantitative studies of the biochemical lesions that develop very early in the course of myocardial infarction. About 500-750 mg. of ischemic tissue can be harvested, which is enough for analysis by many chemical methods. The portion of the infarct in the wall of the left ventricle adjacent to the posterior papillary muscle is heterogeneous, since it contains an unpredictable amount of viable myocardium. The reason for this is the present of anastomoses of fairly large size between the terminal ramifications of the major arterial branches that supply the left ventricle.[11,12] The results of the experiment shown in Table 2 also indicate that some blood flowed into the ischemic area, and that less blood entered the posterior papillary muscle than the lateral wall of the left ventricle. Apparently, the posterior papillary muscle receives most of its blood from the left circumflex coronary artery alone, so that when this vessel is occluded greater ischemia develops in the posterior papillary muscle than elsewhere. Therefore the presence or absence of necrosis in the posterior papillary muscle in these experiments depends upon the vascular pattern in the individual animals. Rarely unusual vascular patterns do occur which may prevent the development of an infarct and constitute a possible source of error. Fortunately, however, such animals are unusual and may be readily identified at operation, because they fail to show cyanosis of the myocardium of the posterior lateral portion of the left ventricle or the characteristic alterations of the electrocardiogram.

Most previous chemical studies of the early changes occurring in myocardial infarction produced by ligation of a large coronary artery have been performed on large samples of tissue taken from the general area of ischemia. Heterogeneous infarct tissue has therefore been used and, though a trend is usually demonstrated in these studies, e. g., an increasing water or sodium content of the infarct, or a decreasing glycogen, creatine, or enzyme content,[10-12] the results usually vary markedly from animal to animal. Use of the homogeneously infarcted posterior papillary muscle for this type of study largely circumvents this difficulty.[13]

Such an infarct, however, is not without disadvantages. The one-hour mortality rate is fairly high, running about 31% in this small series and 23%-25% in Allen and Laadt's larger series,[6] and the longer-term mortality rate is still higher. Deaths are rare in methods involving low ligation of the anterior descending coronary artery,[10] and comparable results would probably be obtained with low ligations of the circumflex artery, but at present the infarct pattern following low ligation of this artery is unknown.

Summary

When the left circumflex coronary artery of dogs is ligated close to its origin, a large infarct develops in the posterior papillary muscle and the posterolateral portion of the left ventricle. In the posterior papillary muscle the infarct is uniform in the sense that practically all the myocardial fibers are dead, but in the wall of the left ventricle the infarct is not uniform and contains many foci of living tissue. Because of the uniform necrosis and the predictable site of the infarct in the posterior papillary muscle, it is admirably suited for correlated chemical and

histochemical analyses of changes occurring during the early stages of myocardial injury due to ischemia or other causes.

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Studies on Distribution and Localization of Potassium in Early Myocardial Ischemic Injury

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Introduction

It has been known for many years that in mammals well-differentiated parenchymal cells usually contain much more potassium than the extracellular fluid which surrounds them, but the mechanisms by which the potassium is maintained at this high intracellular level are still unknown. One possible way of obtaining information about these mechanisms is by a study of the effect of injury upon the potassium content of a tissue composed primarily of potassium-rich cells, such as myocardium, liver, or kidney. This paper describes such a study, using myocardial ischemia in the dog as a model.

Materials and Methods

Two requirements are essential for studies of the early metabolic changes occurring in myocardial ischemia. First, the infarct should be of predictable location, since many of the changes under investigation occur before the infarct has become grossly visible. Second, all the tissue in the infarct should be as uniformly injured as possible so that small shifts of electrolytes can be detected and the results obtained in different animals compared.

The infarct of the posterior papillary muscle recently described by Jennings and Wartman[1] fulfills these requirements. Complete or almost complete necrosis of this papillary muscle can be produced in dogs having a normal arterial distribution by high ligation of the left circumflex coronary artery, and samples of this easily identified area of homogeneous injury are readily obtained for study. Ligation of the left circumflex coronary artery also causes extensive infarction of the posterolateral portion of the left ventricle, but here the infarct contains irregularly located islands of necrotic and normal tissue of variable size which make chemical or histologic analysis difficult.

Thirty adult mongrel dogs were used. They were housed in air-conditioned cages of 70 F and allowed as much food (Borden's Dog Chow) and water as they wanted. Five additional dogs were used as control animals.

Under pentobarbital (Nembutal) anesthesia, the circumflex artery was ligated according to a standardized procedure.[1] Electrocardiograms were taken with a Sanborn direct-writing electrocardiograph before, during, and after the ligation. Animals which did not immediately develop cyanosis in the area supplied by the circumflex coronary artery and elevation of the ST segments in Leads II and III were discarded because previous work had shown that in such animals the posterior papillary muscle might not be involved in the infarct. The chest was closed with silk and the residual air in the left pleural cavity aspirated.

After the desired time interval, the chest was reopened and the myocardium in the circumflex area checked for persistent cyanosis or signs of gross infarction. One or the other of these changes was present in all the dogs with ligations. A sample of blood was withdrawn under oil from the left auricular appendage, and then the heart was removed as rapidly as possible. The posterior papillary muscle with the adjacent portion of the left ventricle was excised and blotted on filter paper. The tip of the muscle was cut off, including all the fibrous tissue associated with the insertion of the chordae tendineae. A thin section through the posterior papillary muscle and the adjacent left ventricular myocardium was fixed in buffered formalin. Other sections from the same area were quick-frozen in isopentane, cooled in liquid nitrogen, and dried under vacuum at —35 C or lower. The remainder of the posterior papillary muscle was resected along the line of its attachment to the ventricular wall and used for chemical analysis.[1]

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Blocks of tissue from the anterior superior portion of the interventricular septum were used for normal left ventricular control tissue, as previous studies had shown it was not involved in the infarct.

Measurement of Potassium.—Tissue samples were placed in tarred weighing bottles, minced with a fine scissors, and weighed immediately on an analytical balance. The samples of posterior papillary muscle weighed between 50 and 300 mg., and those of the left ventricle, between 500 and 2000 mg. The total water content was determined by drying the tissue to constant weight in an oven at 105 C. The fat-free wet weight was determined by extracting the dry tissue for two hours with anhydrous ether U. S.P., followed by a two-hour extraction with petroleum benzine N. F. (petroleum ether). Because the fat-containing solvent was frequently found to contain some electrolyte, the extracts were saved and returned to the tissue after the samples had been dried, weighed, and the fat content determined.[3] The organic solvents were then evaporated off the sample with heat supplied by an infrared lamp. After drying, the samples were quantitatively transferred to a 25×200 mm. Pyrex test tube and extracted with 0.75 N HNO_3 for one hour on a boiling water bath.[3,5] Afterward, the tubes were cooled and the contents diluted to 25 or 50 cc. with 0.75 N HNO_3. After sedimentation or centrifugation the supernatant was analyzed for potassium, using a Beckman DU spectrophotometer with a flame attachment.

The results are reported in milliequivalents of K per kilogram of fat-free wet weight and in milliequivalents of K per 100 gm. of fat-free dry weight. The latter value was calculated by the formula

$$\frac{\text{mEq. K/100 gm. wet weight}}{1-\text{fraction of water}} = \text{mEq. K/100 gm. dry tissue}$$

Potassium Stain.—Potassium was stained in frozen-dried myocardium by a modification of Gersh's cobaltinitrite potassium stain.[4]

Results

Table 1 gives the results in five control animals of analyses for potassium in three different parts of the normal left ventricle: the posterior papillary muscle, the left ventricle beneath the posterior papillary muscle, and the anterior superior portion of the left ventricle. There is no significant difference in the concentration of potassium in these anatomically different portions of the left ventricle, and therefore it is assumed that they are comparable in respect to potassium concentration. The concentration of potassium in the samples of noninfarcted myocardium in the 30 experimental animals was essentially the same (82.48±5.53 mEq. K/kg. fat-free wet wt.).

The results of the analyses for water and potassium contents of the infarct in the posterior papillary muscle and of the control left ventricle are given in Table 2. The general trend of an increase in water content and a decrease in potassium content of the infarct when compared with values for normal left ventricle from the same animal is readily apparent in the raw data. In the last two columns of Table 2 the decreasing potassium content of the posterior papillary muscle infarct is expressed as a percentage of the normal left-ventricle potassium content of the same animal. This method of expressing the data is used because it partially obviates the difficulty of comparing potassium concentrations from multiple animals, in which the range of normal potassium concentration is fairly wide.

The percentage of potassium in the infarct is expressed on both a fat-free wet-weight and a dry-weight basis. This is done because the increase in edema fluid, which has a low potassium content, dilutes the wet-weight tissue potassium in the infarct and

TABLE 1.—*Comparison of Potassium Content of Different Parts of Left Ventricle in Five Healthy Control Animals*

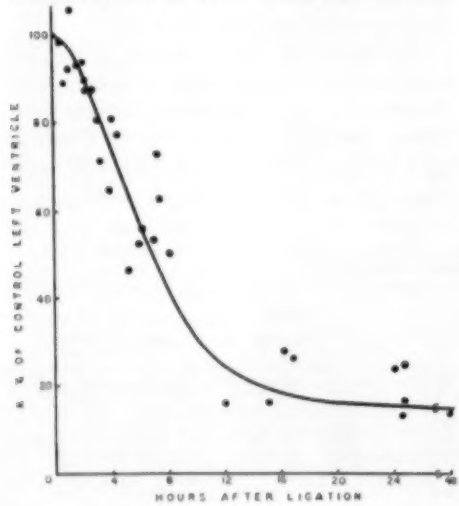
Dog No.	Sex	Weight, Lb.	Potassium					
			Posterior Papillary		Left Ventricle Beneath Post. Pap.		Anterior Superior Left Ventricle	
			mEq/Kg. Wet Wt.	mEq/100 Gm. Dry Wt.	mEq/Kg. Wet Wt.	mEq/100 Gm. Dry Wt.	mEq/Kg. Wet Wt.	mEq/100 Gm. Dry Wt.
143	M	27	79.5	37.0	79.6	36.9	80.6	37.3
145	F	45	84.2	38.7	81.6	37.6	81.7	38.2
146	M	40	82.6	37.1	84.9	38.6	83.7	38.4
147	M	42	80.5	36.6	81.1	36.3	79.2	36.5
148	M	37	88.3	41.9	87.4	41.1	87.8	41.0
Average			83.0	38.3	82.9	37.9	82.6	38.3

TABLE 2.—*Potassium and Water Content of Normal Left Ventricle and Infarct in Posterior Papillary Muscle*

Dog No.	Hours After Ligation	Left Ventricle			Posterior Papillary Infarct				
		H_2O %	K mEq/Kg. Wet Wt.	K mEq/100 Gm. Dry Wt.	H_2O %	K mEq/Kg. Wet Wt.	K mEq/100 Gm. Dry Wt.	% of Normal K	
								Wet Wt.	Dry Wt.
115	0/20′	77.9	79.1	35.7	76.7	81.6	35.0	103.2	98.1
150	0/40′	77.5	86.0	37.7	78.4	73.3	33.5	85.2	88.7
149	1/	79.3	82.8	39.4	79.3	76.4	36.1	92.3	91.8
80	1/17′	78.5	79.3	36.8	81.2	74.9	39.0	94.4	106
140	1/38′	79.0	81.5	39.6	81.2	69.1	36.8	84.8	92.8
110	1/56′	78.4	82.0	38.0	80.3	69.4	35.3	84.7	92.9
108	2/10′	77.7	86.8	39.1	79.6	71.3	35.0	82.2	89.4
107	2/12′	78.0	79.7	36.1	79.3	66.3	31.4	83.3	86.9
114	2/38′	78.8	82.7	38.9	80.9	64.5	33.8	78.0	86.8
106	3/5′	77.5	81.7	36.3	78.8	62.0	29.2	75.9	80.6
104	3/13′	78.9	77.8	37.7	80.6	52.2	26.9	67.1	71.4
127	3/45′	78.8	80.4	37.9	84.8	37.2	24.5	46.3	64.7
121	3/57′	77.0	84.5	36.7	80.5	57.6	29.5	68.2	80.4
101	4/15′	77.6	79.2	35.4	77.9	61.3	27.7	77.4	78.3
132	5/10′	79.5	86.5	42.1	80.6	38.5	19.6	44.0	46.5
123	6/	78.8	84.2	39.7	81.3	39.0	20.9	46.5	52.7
142	6/10′	79.3	98.7	47.5	81.5	49.5	26.8	50.2	56.3
100	7/5′	78.1	84.3	36.7	80.1	38.9	19.5	46.1	53.3
137	7/15′	78.4	81.8	37.9	80.3	54.1	27.5	66.2	72.6
141	7/20′	79.7	76.6	37.7	81.6	44.0	23.9	57.5	63.4
98	8/7′	78.1	82.1	37.5	81.3	35.0	18.7	42.7	50.0
93	12/	77.7	79.5	35.5	83.9	9.2	5.7	11.5	16.1
144	15/8′	77.6	86.5	39.0	82.5	11.4	6.3	13.1	16.3
151	16/7′	78.1	88.6	39.5	82.6	20.0	11.0	12.4	27.8
153	16/45′	77.9	90.0	39.8	81.6	18.9	9.9	11.0	25.4
135	24/8′	78.2	77.6	35.5	82.2	14.6	8.2	18.8	23.1
82	24/30′	80.6	73.9	38.1	83.4	8.1	4.9	11.0	12.8
79	24/45′	78.5	87.8	40.9	82.3	11.6	6.6	13.2	16.0
86	24/45′	78.0	73.4	34.3	79.2	17.7	11.6	11.6	24.8
71	48/30′	78.1	92.5	41.7	81.5	10.4	11.2	11.2	13.5
	Mean	78.38	82.9	38.3					
	S.D.	±0.77	±5.4	±2.58					

gives a lower potassium concentration than actually occurred due to potassium loss from injured fibers. The fat-free dry-weight data eliminate this dilution phenomenon, and for this reason are considered to be a more accurate representation than the wet weight of the changes in potassium content of the fibers within the infarct.*

Fig. 1.—Graph showing the relationship between potassium content of infarct and duration of injury. The potassium content in this instance is calculated on a dry-weight basis and plotted as a percentage of the dry-weight potassium of the control left ventricle from the same animal.



The decreasing potassium content of the infarct tissue, based on dry weight, is plotted against duration of injury in Figure 1. The resultant curve is an approximation drawn to fit the data. It shows that, after about 90 minutes, potassium rapidly disappears from the infarct, until levels close to that of the extracellular fluid are reached 12 hours after ligation. The only difference between this curve and that shown in Figure 2, which is based on wet-weight data, is the fact that the wet-weight potassium concentration is always lower when compared with normal than the dry-weight data from the same animal. In addition, no initial period of slow loss is apparent in the wet-weight data.

* The dry weight method of calculation is also inaccurate after the effects of the injury have fully developed, because substances such as glycogen, amino acids, and enzymes, which contribute to the dry weight, begin to leave the infarct.[6]

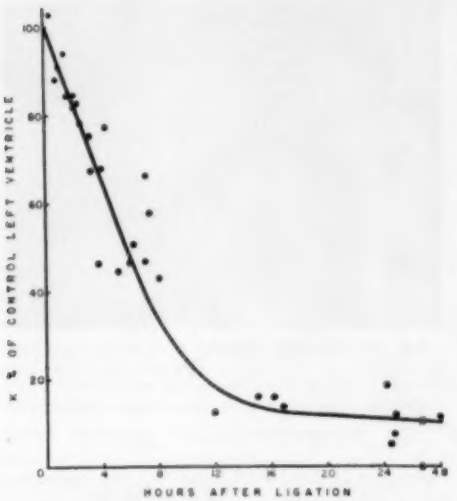


Fig. 2.—Graph showing the same data as Figure 1 except that the potassium content is calculated on the basis of the fat-free wet weight. Note the rapid drop in potassium content of the infarct in Figure 2 as compared with Figure 1.

Localization of Potassium.—This was studied in histological sections of tissue stained by a modification of Gersh's potassium stain. In this method, potassium is precipitated by cobaltinitrite at the site it theoretically occupied in the muscle fiber when the sample was quick-frozen with liquid nitrogen. A dense precipitate of granular golden-brown potassium cobaltinitrite forms and is easily identified with either the light or the phase-contrast microscope. The distributions of potassium within the normal muscle fibers of the left ventricle of all the animals were similar. Figure 3 and 5 are typical of normal cardiac muscle and show the precipitate evenly deposited throughout the fiber.

A decrease in the amount of potassium cobaltinitrite precipitated within the fiber was apparent in the infarct in the posterior papillary muscle at the end of six hours and by eight hours was quite obvious (Fig. 4). Sections of the infarct after 24 hours (Fig. 6) showed that there was almost no potassium in the fibers. There was no particular pattern to the loss of potassium, and at the time intervals studied it was not differentially distributed within the fiber.

Comment

In these experiments, the early changes occurring in the potassium ion content of myocardial fibers subjected to irreversible ischemic injury have ben studied in dogs. Uniform ischemic necrosis was produced in the posterior papillary muscle of the left ventricle by high ligation of the left circumflex coronary artery, and samples of ischemic tissues were taken at various time intervals and analyzed by a combination of chemical and histochemical methods.

The results of the chemical analyses of the injured tissues, which are shown in Table 2 and Figure 1, indicate that during the first 60 to 90 minutes after ligation a

Fig. 3 (Dog 98).—Frozen-dried normal section of left ventricle. Note the even distribution of the precipitate of potassium cobaltinitrite. The precipitate is so dense that the cross striations of the fibers are obscured. × 1290.

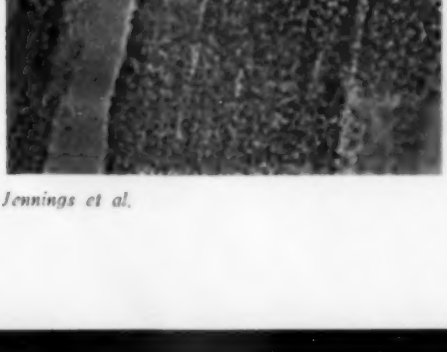


Fig. 4 (Dog 98).—Section of posterior papillary muscle infarct (eight hours after ligation) from the same animal. Note the decreased density of the precipitate, allowing one to see the cross striations. × 1290.





Fig. 5 (Dog 79).—Section of normal left ventricle, showing the distribution of potassium in uninjured left ventricle. Note the similarity to normal left ventricle from Dog 98, shown in Figure 3. × 1290.

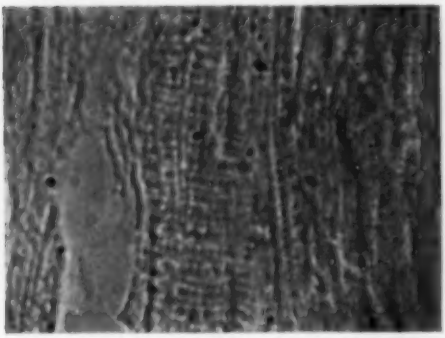


Fig. 6 (Dog 79).—Section of posterior papillary muscle infarct 24¼ hours after ligation. Note that the fibers are free of precipitate except for a few scattered large granules of potassium cobaltinitrite. × 1290.

small drop in the concentration of potassium occurred and that after this time the potassium ion content decreased at a more rapid rate. By the end of 12 hours the concentration of intracellular potassium was about the same as that of the extracellular fluid. Histochemical methods, using cobaltinitrite, showed no change until about six hours after the onset of ischemia, when a perceptible decrease in the density of the precipitate was noted. At this time chemical analyses show a 50% loss of intracellular potassium. By 12-15 hours, however, the histochemical method also showed complete loss of potassium. Thus the results of both methods agree on the time of final disappearance of potassium from the ischemic myocardial fibers. It is interesting to note that the histochemical studies of normal myocardial fibers showed the potassium ions distributed diffusely in the cytoplasm without regard to any specific cellular structures, such as mitochondria, sarcoplasm, contractile fibrils, or intercalated discs. Following injury, potassium left the cells in an indiscriminate manner, without regard to their structure.

The changes in fiber potassium content in the posterior papillary muscle infarct are clearly established for the period starting about two hours after the onset of ischemia. However, the initial changes in potassium content are more difficult to evaluate (Fig. 1). This is because of the difficulties inherent in any method involving the measurement and comparison of small changes in ion content in biologic material obtained from multiple animals. It is believed that more accurate information about the changes occurring during the first two hours after ligation probably cannot be obtained by the direct method of approach unless a technique is developed for taking multiple samples from a single animal. The data obtained in the experiments reported in this paper indicate, but do not prove, that potassium leaves the injured fibers at a slow rate during the first 60 to 90 minutes after the onset of the injury.

Assuming that the curve shown in Figure 1 is accurate and that the time required for diffusion of potassium from the infarct does not appreciably delay the appearance of potassium loss from the injured myocardial fibers, one could hypothesize that whatever mechanism is responsible for the retention or concentration of potassium within the fiber fails completely after about 60 to 90 minutes of in vivo ischemia and that potassium, therefore, is rapidly lost from the myocardial fiber at this time. This might be due to failure of energy-producing mechanisms involved in maintaining the concentration of potassium within the cell, or to delayed release of potassium-containing proteins or other slowly diffusible substances from the fiber. Whatever the cause, detailed

study of the metabolic patterns of the injured tissue during this interval might lead to identification of the mechanism involved.

The results of our experiments suggest little or no change in potassium content of the fibers during the first few minutes after ligation. However, some loss of potassium from the ischemic fiber must occur during the first 10 minutes of ischemia, because during this time interval several investigators [6-8] have demonstrated a definite rise in the level of plasma potassium in blood draining from ischemic cardiac muscle in dogs with infarcts. This same phenomenon has also been demonstrated in blood draining from ischemic skeletal muscle in cats.[14] Harris and associates [6] showed a later rise in coronary plasma potassium 3-4 hours after ligation, which correlates well with the data shown in Figures 1 and 2, and the initial rise shown by the above experiments [6-9] therefore probably represents a small loss from many fibers during the first 10 minutes of ischemia. Perhaps a small portion of the potassium is readily diffusible and leaves the fiber almost immediately after the onset of ischemia, while the bulk of the potassium is more firmly bound or is still being concentrated, and does not begin to diffuse from the cell until 60 to 90 minutes after ligation.

Further evidence that the immediate potassium-loss phase described above [6-9] may not be of long duration and of great quantitative significance in relation to direct tissue analysis is obtained from the experiments of Lowry, Krayer, Hastings, and Tucker [10] on the effect of anoxia on myocardial electrolyte levels in canine heart-lung preparations, in which only a small drop in tissue potassum concentration was demonstrated an hour after the onset of almost complete anoxia.

The ion relationships in isolated invertebrate skeletal muscle preparations have been studied by many investigators, but it is difficult to apply the results of these experiments to our studies on the heart. Horton's [11] work on the effect of injury on frog sartorius is of the most interest. He showed that this muscle loses up to 25% of its potassium after a 30-minute soak in Ringer's solution and that a 48-hour soak only results in the loss of another 50% of the total potassium. An adequate explanation of why 25% of the sartorius potassium is not leached from the muscle after this long time interval might cast some light on the relationships of potassium ion to the intracellular contstituents of the fiber.

Summary

The pattern of loss of ionic potassium from irreversibly injured myocardial fibers has been studied in a homogeneous infarct in the posterior papillary muscle of the left ventricle of dogs. Chemical analysis of the infarct showed about 10% loss during the first 60 to 90 minutes, but after this time potassium rapidly disappeared from the injured fibers. By the end of 12 hours the intracellular potassium had fallen nearly to the level of this ion in the extracellular fluid.

Histochemical studies were made of both normal and ischemic myocardium, using a cobaltinitrite method. Normally potassium was distributed evenly throughout the fiber and without demonstrable relation to intracellular structures. After the onset of ischemia no change in the distribution of potassium was detected until six hours, when a qualitative decrease in the amount of potassium cobaltinitrite became apparent. At the end of 12 hours little or no potassium could not be demonstrated in the fibers, thus confirming the results of the chemical analysis.

Dr. William B. Wartman gave advice and encouragement, and Lore Veit, Mae Krivich, and Zigmund Zudyk furnished technical assistance.

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Certain Dietary Effects on the Serum Cholesterol and Atherogenesis in the Rat

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Man and the albino rat resemble each other in major dietary requirements and cholesterol metabolism. Therefore the rat would seem to be an ideal animal for the experimental study of factors involved in atherosclerosis, especially since this species is considered to be even more resistant than man to the development of atheromatous coronary artery lesions. Such lesions have recently been observed in rats fed an adequate synthetic diet containing physiological amounts of cholesterol, fat, and protein.[1] Their histological character and slow development suggest a similarity to the early human disease. These studies were undertaken to examine the importance of certain elements of this diet, popularly considered to be important in human coronary atherosclerosis.

In the previous experiment atheromatous lesions were produced in the coronary arteries of rats fed a synthetic diet high in lard and choline and containing cholesterol. Brown and Page [2] produced a much greater degree of hyperlipemia with antithyroid treatment and 4% dietary cholesterol without observing atheromatous lesions in the rat. The explanation for these discordant results may reside in differences in the strain of white rat, the age and previous nutrition of the animals used, or the method of examining the arteries, or in dietary elements used—cholesterol presumably excepted. In the experiments described here the role of various dietary ingredients in the pathogenesis of coronary atheromatous lesions was evaluated, using younger rats. Young adult rats were fed an atherogenic diet, which was modified after one year to determine whether the lesions could be reversed. An evaluation was also made of whether serum lipid levels correlated with the presence of lesions, and whether the lesions would be observed in animals fed a low-fat, low-choline, low-cholesterol diet, which would induce a minimal degree of hyperlipemia. A second experiment was then performed to investigate the possible role of deficiency of methionine or α-tocopherol on this hyperlipemia.

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Method

One hundred 12-week-old albino rats of the Sprague-Dawley strain, weighing 250 to 300 gm., were placed on the diet fed Group III in the previous experiment,[1] hereinafter the experimental diet (E); 10 animals were fed a control diet (C), similar to Diet E except for a reduction in fat, choline, and cholesterol; and another group of 10 rats was fed Purina Laboratory Chow. The animals were kept in wire-bottomed cages in a thermostatically controlled room with a maximum of seven rats per cage. They were maintained on these diets ad libitum; diet consumption was estimated daily and body weight measured weekly.

Eighty-six animals survived one year on the original diets, at which time those on the experimental diet were reorganized into five groups matched as to body weight. One group (E-E) was continued on the experimental Diet E; a second group (E-D) was transferred to a similar diet (D), varying only in its low protein level; a third group (E-F) was placed on a Diet F, similar to Diet E except for the inclusion of a lipid-poor residue of mammalian brain shown to be hypocholesteremic in cholesterol-fed chicks[4] and man[5]; a fourth group (E-C) was placed on Diet C, and the fifth group (E), which had been on Diet E for one year, was killed. The

TABLE 1.—*Percentage of Variable Constituents in the Diet* of Groups Described*

Group	C	D	E†	F
Lard	8.0	30.0	30.0	30.0
Dextrin	62.0	52.5	38.75	33.75
Casein	15.0	5.0	18.75	13.75
Choline	0.05	0.625	0.625	0.625
Cholesterol	0.00	0.3125	0.3125	0.3125
Brain fraction	....	....	....	10.0

* The constitution of the supplementary salt mix, vitamin mix, and other ingredients and the source for all materials were listed in the previous paper.[1]
† Diet III in previous paper.[1]

two control groups were continued as before on Chow and the control Diet (C), until the termination of the experiment (18 months from the onset), when all surviving animals were killed by exsanguination under ether anesthesia. The variable constituents in the Diets C, D, E, and F can be seen in Table 1. A salt mix, a vitamin mix, non-nutritive edible cellulose (Ruffex), and percomorph liver oil (Oleum Percomorphum*) were added, as in the previous experiment.

The rats were bled from the tail vein, under ether anesthesia, at intervals of one to three months throughout the experiment, the blood being pooled in groups of 10 animals or less for the first year and according to the dietary groups thereafter. The serum derived from these pooled samples of blood was analyzed for serum cholesterol, phospholipid, and, when sufficient serum was available, total lipid by the methods outlined by Bloor.[9]

All animals, whether killed or dying in the course of the experiment, were autopsied. Frozen sections of the heart (transverse and frontal), aorta, kidney, and liver were stained with Sudan III, and paraffin sections of selected tissues were stained with hematoxylin and eosin, as previously described.[1]

In the second experiment, four groups (I-IV) of 10 rats were again started on Diet E, but half (groups III and IV) were given a supplement of DL-methionine 0.5%. After a period of six months the use of percomorph liver oil was discontinued as a source of the fat-soluble vitamins, and, instead, all animals were given crystalline vitamin A (0.002% by weight), while half of the rats (Groups II and IV) received an additional supplement of α-tocopherol (0.02% of the diet), or an estimated 3 mg/rat/day. This diet was continued then for six more months and the animals killed. Blood samples were obtained from the tail, using secobarbital (Seconal) anesthesia, at 2, 6, 9, and 12 months. This time the serum cholesterol of individual rats was determined by the ferric chloride method of Zlatkis.[6] Otherwise, the methods were the same as described above.

* Supplied by Mead Johnson & Company.

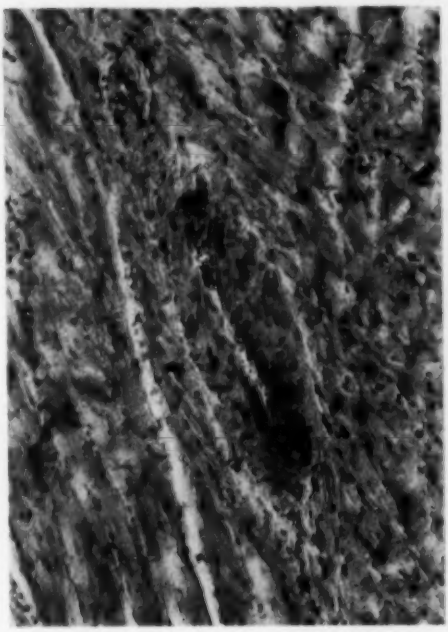


Fig. 1.—Medium-sized coronary artery of a rat fed the experimental ration for 12 months (E). The sudanophilic material in the intima appears black in the photograph. Frozen section; Sudan IV; reduced to 60% of mag. × 260.

Results

First Experiment.—Three months after the start of the experiment (when the rats were 6 months of age) a pneumonitis, presumably the "endemic pneumonia," so well described by Nelson,[7] or the "chronic murine pneumonia," discussed by Innes et al.[8] became manifest in a few animals. In spite of the administration of oxytetracycline (Terramycin)† to the colony, 15 mg. per rat per day for two months, it caused a persistent level of morbidity and mortality for the duration of the experiment.

The animals gained weight slowly for the first six months, though the weight gain was greater in the Chow-fed controls. Thereafter a gradual steady decline in average weight began. The low-protein diet (D) precipitated rapid weight loss and

† Supplied by Dr. W. Allen Wright, of Pfizer Laboratories.

death in two to three months. (One blood sample was obtained from these animals after the diet was shifted, but all died before the time of killing, so that no terminal blood samples were obtained.) At about this same time, in the 15th month of the experiment, the remaining animals (excepting the Chow group) developed a roughening of the coat and obvious wasting and feebleness of the hind limbs. The latter simulated a paralysis, the animal dragging his hind quarters about the cage.

Histopathologic Findings: The incidence of lipid-containing coronary artery lesions in the various dietary groups was comparable to that previously reported; however, these lesions were less widespread, smaller, and less heavily laden with lipid than those seen in the earlier experiment.[1] They were unequivocal and of the same general character (Fig. 1). No arteries other than the coronary arteries were apparently involved in the process. In the series of 35 animals dying of pneumonitis or other causes before the 12-month date of killing, only 2 showed early subintimal sudanophilic changes after about 9 months on the experimental diet.

An unanticipated finding was that the combined incidence of atheromatous lesions of the coronary arteries was identical in the groups on the experimental diet (E and E-E) and those on the control diet for six months or longer (C-C and E-C). In both categories lesions developed in about 20% of the animals, an incidence comparable with that found in the earlier experiment,[1] though the animals continuously on Diet C showed arterial lipid infiltration without the proliferative changes seen in the other groups. The incidence in the coronary arteries of rats fed on the low protein or the supplement of brain fraction could not be said to vary significantly from this, though it can be seen from Table 2 that the intensity and frequency tended to be less in the latter group. Not 1 of the 10 animals fed Purina Laboratory Chow was found to have lesions. This agrees with a much broader autopsy experience with old Chow-fed animals, where only one rat in several hundred has ever been found with lipid in his coronary arteries. On the other hand, Malinow et al.[9] have recently reported a fairly high incidence of fat-containing arterial lesions in rats maintained on a mixed diet, including bread, milk, meat, and vegetables.

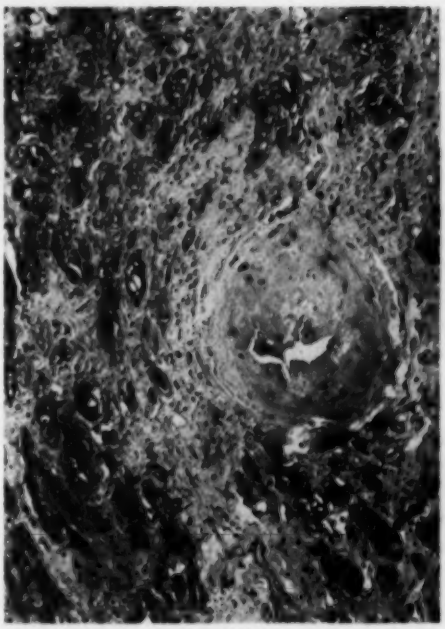


Fig. 2.—Section from the heart of a rat receiving Diet E for 18 months. The thickened coronary artery shows severe medial degeneration, as well as distortion and swelling of the intima, accompanied by vacuolation. The surrounding heart muscle is partially destroyed, with intervening myofibrosis and a mild subacute to chronic inflammatory cellular infiltration. Paraffin section; hematoxylin and eosin; reduced to 60% of mag. × 250.

TABLE 2.—*Incidence of Animals with Coronary Arterial Atheromatous Lesions in Each Group Autopsied After at Least One Year on Diet*

Diet Group	No.	No. with Lesions	Average Intensity	No. with Pneumonia
Chow	9	0	0	8
C–C	6	2	1	0
E	18	3	2	14
E–E	12	3	2	7
E–F	12	2	1½	7
E–D	13	4	1¾	9
E–C	14	2	1½	6

The coronary artery lesions were characterized by degeneration of the intima with

frequent incorporation of lipid-staining material (Fig. 1). Occasionally the walls of the large and medium-sized coronary arteries were thickened by a pale, vacuolated material, which stained pink with hematoxylin and eosin and which distorted the intima dramatically (Fig. 2). In some areas the medial musculature appeared to be partially replaced by this eosinophilic material. The frequency of atheromatous lesions would actually be greater than recorded here if such lesions were included in the count. Many of these lesions encroached upon the lumen, as would a plaque. Less frequently, focal arterial endothelial proliferation was present. Lipid infiltration of the endocardium was noted rarely.

A chronic myocarditis was more frequently seen, varying from mild to severe degree and characterized by focal replacement of myocardial fibers by fibrous tissue and slight round-cell infiltration (Fig. 2). The chronic renal changes described in the previous experiment [1] were surprisingly infrequent in these animals. They consisted of dilatation and obstruction of renal tubules with dense eosinophilic material, accompanied by atrophy of associated portions of the nephron and little vascular or inflammatory change. An occasional pancreas showed varying degrees of focal acinar atrophy. The one consistent difference between Chow-fed and experimental animals was the presence of testicular atrophy in the latter. This varied from moderate depression of spermatogenesis to complete atrophy of the testicular tubules (Fig. 3). The only microscopically normal gonads occurred in those animals dying after only a few months on the experimental diet. Associated with this, skeletal muscles from many rats fed the synthetic diets showed

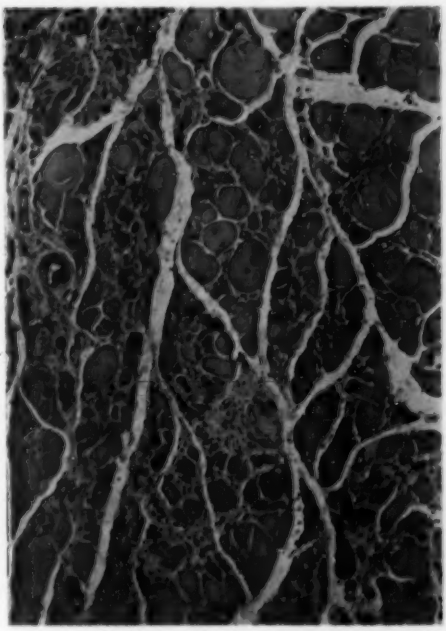
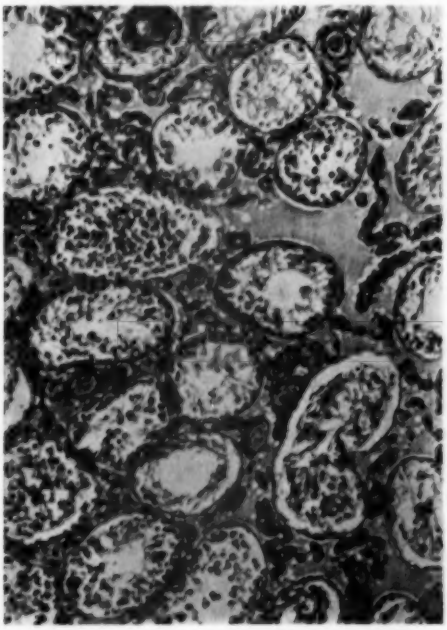


Fig. 4.—Right quadriceps femoris muscle with focal atrophy and hypertrophy. These changes and the relative increase in fibrous tissue and inflammatory cells are characteristic of the histologic changes seen in skeletal muscles from many of the animals fed the synthetic rations for 18 months. Paraffin section; hematoxylin and eosin; reduced to 60% of mag. × 175.

Fig. 3.—Testis representative of those from animals fed the synthetic rations for 8 to 10 months or longer. Note the remarkable atrophy and simplification of the seminiferous tubules, with desquamation of some of the spermatogonia. Evidence of interstitial edema was also seen frequently. Paraffin section; hematoxylin and eosin; reduced to 60% of mag. × 150.



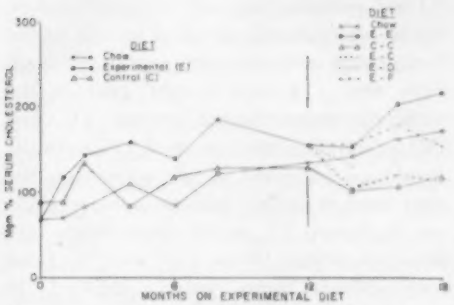


Fig. 5.—Average of pooled serum cholesterol determinations in the various dietary groups indicated in the legend. The vertical line indicates the reorganization of surviving animals into the new dietary groups, as described in text.

large areas of focal atrophy and slight compensatory hypertrophy, with a relative increase in fibrous tissue and a slight subacute to chronic inflammatory response (Fig. 4).

Serum Lipid Studies: The serum cholesterol gradually rose in the course of the first four months of the experiment, tended to fluctuate as more of the colony developed pneumonitis, showed a rise, perhaps attributable to oxytetracycline, and returned essentially to the four-month level at the end of 12 months (Fig. 5). After the 12-month survivors were redistributed, the serum cholesterol of the group placed on the control diet (E-C) fell to near-control levels, while that of the Chow group and other dietary groups, except Group E-D, tended to rise during the last four months of the experiment. In view of the findings at autopsy, this rise may have represented the development of complications of advancing age, such as renal disease, in certain individual rats within these groups. Total lipid and lipid phosphorus determinations on these pooled sera tended to rise with the serum cholesterol.

Table 3 shows the mean terminal serum lipid values in each group killed at 12 and 18 months. It can be seen that, though the Chow animals had fairly high levels of total lipid and cholesterol, they also had a fairly high phospholipid level, so that the cholesterol-lipid phosphorus (C/P) ratio was lower than in any other group. The animals terminally on a control diet (C-C and E-C) showed the lowest serum cholesterol level but a somewhat higher C/P ratio than the Chow group. Group E-F presented a cholesterol level almost as high as did Group E-E, but the C/P ratio was only moderately elevated.

On examining the terminal lipid values of the individual rats having arterial lesions, we found that no rat with a terminal C/P ratio less than 22 developed arterial lesions, though four with ratios of 22 or higher did so. Unfortunately, ratios were not available on the other rats with lesions, but this finding tends to confirm the earlier work.[1]

Certain interesting observations were made regarding the "chronic murine pneumonia" which so universally complicates chronic rat studies.[7,8] The pneumonitis was accompanied by tracheobronchitis and bronchiectasis, which seemed to precede the development of a suppurative pneumonia. Though dietary influences have been demonstrated in bacterial disease in rats,[10,11] we were not prepared for the poor resistance to this disease manifested by the animals on the experimental diet, where an incidence of about 75% prevailed at the age of 15 months. The lungs of animals on Chow were almost universally infected, but these animals tolerated the disease well, only 3 out of 10 succumbing to the disease in 18 months. These animals gained the most weight initially and, in the latter months, lost less than any other group. The animals

TABLE 3.—*Terminal Lipid Values in Surviving Groups at Time of Killing**

Diet Group	Total Lipid, Mg/100 Cc.	Cholesterol Mg/100 Cc.	Lipid Phosphorus, Mg/100 Cc.	C/P
Chow	684	173	8.6	20.8
	(4)	(7)	(5)	(5)
C-C	363	125	5.6	22.1
	(3)	(4)	(4)	(4)
E	447	154	5.9	26.5
	(8)	(10)	(10)	(10)
E-E	596	218	8.3	26.4
	(5)	(6)	(6)	(6)
E-F	412	164	7.7	22.4
	(3)	(7)	(5)	(5)
E-C	354	115	5.5	21.7
	(5)	(7)	(7)	(7)
E-D	All animals died; none killed.			

* The number in parentheses indicates the number of animals from which the average is derived.

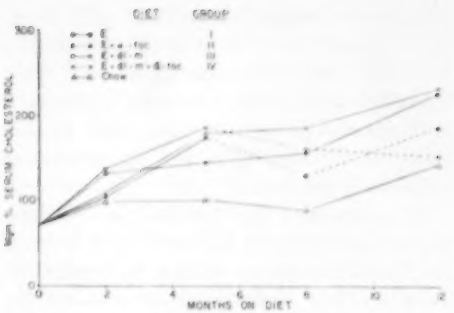


Fig. 6.—Mean serum cholesterol levels on the individual rats of each of the indicated dietary groups.

in Group C-C, although caged near the Chow group, were free of the disease, with one exception. The 12-month necropsy group (E) had a higher incidence than the 18-month groups, all of which were about equally affected (65%). There was no correlation between the development of atheromatous lesions and the presence or absence of pneumonitis at death or time of killing, and the average weight at this time was not significantly lower in the animals having pneumonitis.

Second Experiment.—From the results of the first experiment it was apparent that neither exclusion of the cholesterol supplement from the diet, with reduction of fat and choline to more modest levels, nor late reduction of protein level or supplementation with a hypocholesteremic brain preparation made any dramatic difference in the incidence of atheromatous lesions consequent to the basic diet. The likelihood that this diet is deficient in vitamin E and perhaps suboptimal in methionine led us to do the second experiment.

Food consumption, and therefore caloric intake, was virtually identical in each of the four groups until the eighth month, although there was a greater weight gain in the methionine-supplemented groups (II and IV). Supplementation with vitamin E caused an increased weight gain also, the most marked weight increment being present in Group II, which then closely approached Group IV in final weight. Food consumptions were nearly identical in Groups III and IV, though Group IV gained more weight than Group III once α-tocopherol supplementation was started.

The average serial cholesterol changes in the groups under discussion may be seen in Figure 6. The mean serum cholesterol level at five months was significantly higher ($P<0.01$) with methionine than without it, though by 12 months this difference had disappeared. Late supplementation with α-tocopherol caused a fall in serum cholesterol in three months, which was highly significant ($P<0.01$) in Groups II and IV when compared with Groups I and III. Terminally the difference was even greater in the methionine-supplemented group, though only maintained within Group II. Group IV, which received supplements of both methionine and α-tocopherol, most closely approached the Chow-fed animals in terminal serum cholesterol level.

Table 4 shows the relative frequency of coronary atheromatous lesions in each of the five groups examined. No lesions were noted in the animals fed Chow for a year, and this agrees with previous experience. Methionine or late vitamin E supplementation did not abolish lesions; in fact, the

TABLE 4.—*Incidence of Coronary Artery Atheromatous Lesions in Each Dietary Group After Twelve Months*

	Diet Supplement		Animals Studied				Coronary Arteries Seen		
					Lesions Seen			Lesions Seen	
Group	DL-Methionine	α-Tocopherol*	Final Wt., Gm.	Total	No.	%	Total	No.	%
Control	Chow			10	0	0	106	0	0
I	—	—	382	10	2	20	156	5	3
II	—	+	560	12	3	25	153	3	2
III	+	—	503	11	2	18	108	2	1
IV	+	+	570	8	3	37	103	4	4

* Supplement added only after six months on diets.

highest percentage of lesions was seen in the group receiving both supplements, though this is certainly not statistically significant.

Testicular weights were recorded and found to be about half as great in Group I as in the other three groups. On microscopic examination, the liver was equally fatty in all groups. There was no gross evidence of muscular dystrophy in any of these animals, and there was no evidence for vitamin E deficiency in the myocardium, though histologic studies of testes and skeletal muscle were not carried out. These animals were not as old at time of killing as those in the first experiment, and only the rats in Group I presented a high incidence (80%) of pneumonitis, the other groups having less than 50% involvement. It may well have been this disease that produced the average terminal weight loss in Group I.

Comment

The most important fact derived from these experiments was that the lard-casein diet, originally used by Wissler, Eilert, et al.[1] to produce atheromatous, lipid-laden lesions in rats, maintained this ability even though (1) the only source of dietary cholesterol was the small amount of lard (reported to have only 0.108% cholesterol [13]), (2) the fat intake was reduced to 10% by weight, and (3) the high choline level was made more moderate. The fact that supplementary dietary cholesterol is not necessary for the development of these lesions is in agreement with preliminary results previously reported.[12]

The development of hindlimb muscle weakness, testicular atrophy, and a nonspecific myocardial fibrosis all suggested the presence of a chronic vitamin E deficiency. A casein-lard diet was originally used for the production of vitamin E deficiency in suckling rats [14] or other animals.[15] There are other reports of vitamin E deficiency induced in adult rats reared on an adequate diet for the period of rapid growth,[16-19] but in none of these experiments have arterial lesions been noted. Heart muscle lesions have been reported in vitamin E-deficient mice, also deficient in vitamin K,[20] and in vitamin E-deficient guinea pigs.[21] In the latter instance adventitial thickening of the coronary artery walls was also described.

It is apparent from our results that treatment of our animals with α-tocopherol from the sixth month of this diet did not prevent the development or progression of the lesions. This cannot be considered conclusive evidence against vitamin E deficiency as a causative factor, inasmuch as the brown discoloration of the uterus [18] and, to some extent, the muscle dystrophy of chronic avitaminosis E are much more easily prevented than reversed by vitamin E administration.[22] On the other hand, several observers [23-25] have noted the absence of any improvement, if not actual exaggeration, of experimentally produced atherosclerosis by adding a dietary supplement (often excessive) of α-tocopherol to their regimen. Furthermore, a more recent experiment performed by one of us (R. W. W.),[26] employing large groups of rats, has demonstrated conclusively that α-tocopherol supplementation of the high lard-casein ration will not prevent the development of the coronary atheromatous lesions in rats over a nine-month period.

Another possibility was that a mild methionine deficiency was important in the production of lesions. Mann and co-workers have shown that a methionine deficiency in rats will produce a hypercholesteremia [27] and will produce atheromatous lesions in Cebus monkeys.[28] It has been shown [29] that a diet in which casein is the sole source of protein may be suboptimal in methionine content for rapid growth in the rat. The findings reported here indicate that, while supplementation with methionine does permit better growth and a healthier animal under our dietary conditions, it does not prevent the hyperlipemia or the coronary arterial lesions. While a more definite methionine deficiency may produce a hypercholesteremia and, in monkeys, arterial le-

sions, this does not appear to explain the lesions seen in rats fed this casein-lard diet.

As far as the hypercholesteremia is concerned, our data indicate that methionine supplementation of the experimental Diet E causes a significant rise in serum cholesterol. This is just the opposite of the effect of methionine supplementation seen by Mann and co-workers [28] in their methionine-deficient monkeys fed soy bean α-protein.

Supplementation with vitamin E in these deficient rats did significantly increase body weight and lower the serum cholesterol, and the Group IV which received both α-tocopherol and methionine had cholesterol levels restored to virtually the same level as the Chow-fed animals of similar age. Since both these supplements improved food utilization and body growth, a decrease in cholesterol or fat consumption did not account for these changes in cholesterol level. It is perhaps worthy of mention that the diet of Filios and Mann [27] was also deficient in α-tocopherol, percomorph liver oil being the only source of fat-soluble vitamins. The fact that methionine supplementation tended to correct the hypercholesteremia on their soy bean α-protein diet is reminiscent of the apparent synergism between α-tocopherol and methionine-cystine deficiency in the production of acute necrosis of the liver.[30] It is possible that the hypercholesteremia they observed was due in part to a vitamin E deficiency and thus was not completely corrected by methionine supplementation alone.

Summary and Conclusions

Investigation of certain dietary elements used in the original production of atheromatous lesions in rats has led us to the following conclusions:

1. The development of coronary arterial atheromatous lesions by the ad libitum feeding for a period of at least one year of a diet containing casein as the protein source, and lard with percomorph liver oil as the fat source is confirmed.

2. The incidence of lesions does not depend upon the presence of a high level of fat or cholesterol in the diet.

3. Efforts to alter the incidence of lesions by lowering the level of protein or adding a supplement of a hypocholesteremic agent were unsuccessful.

4. Though this diet is deficient in vitamin E and treatment with α-tocopherol tends to improve nutrition and correct the hypercholesteremia, the lesions are clearly not reversed or prevented by α-tocopherol therapy.

5. Methionine supplementation alone does not influence the frequency of lesions, and it exaggerates the hypercholesteremia.

6. The hypercholesteremia is reduced to the level seen in Chow-fed controls when both methionine and α-tocopherol are included in the experimental diet.

Charles Straley, Irene Carr, and Kathryn Soules gave technical assistance in this work.

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Bronchogenic Carcinoma

A Statistical Analysis of Two Hundred Ninety-Six Cases with Necropsy as to Relationships Between Cell Types and Age, Sex, and Metastasis

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In addition to the alarming increase in bronchogenic carcinoma in general—perhaps the most remarkable oncological feature of the present century—there have been concurrent changes in the comparative incidence of the major cell types of this disease. To varying degrees, these cell types have their own special associations with the incidence of bronchogenic carcinoma as to sex and age, and with potentiality for, and pattern of, metastasis. It is becoming apparent that all of these changes may be related, through their dependence on cell type, to the activity of varying extrinsic causal factors. Statistical studies of bronchogenic carcinoma as to the interrelationships of age incidence, sex incidence, cell type, and occurrence and distribution of metastasis always have had obvious clinical applications in estimating presumptive curability or palliation by operation or irradiation. Such studies are now of increasing importance in etiological investigations.

As will appear presently, the necessary subdivision of cases into subgroups on the combined bases of cell type and sex results in classes which vary greatly in numbers. Some of these, when dependent on the material from a single institution, prove to be too small to inspire confidence. This is true particularly of the category of squamous-cell bronchogenic carcinoma in females. It is believed to be of both current and future usefulness to publish the results of even relatively small series in such a way that they will be helpful to those who undertake summation and analysis of the figures from many sources. Such has been the purpose in developing this report.

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Material

All cases of bronchogenic carcinoma with complete necropsies at the University of Michigan Hospital were surveyed in the course of this study. The check lists indicated a total of 313 cases. Critical review of both clinical and necropsy reports resulted in the deletion of 17 cases, chiefly because it was impossible to be certain that a far-advanced and widely generalized neoplasm was beyond doubt primary in the bronchial tree. This left 296 cases for analysis and tabulation.

The microscopic sections from each necropsy were available for a critical review of the cell type as originally diagnosed. For those cases in which thoracotomy had preceded death and no residual neoplasm was found at necropsy, sections from the surgical material were used to establish classification.

While the 296 usable cases were derived from 14,000 necropsies performed over a period of almost 60 years, most of them had been accessioned rather recently. This was true, in part, because necropsies were being performed in constantly increasing numbers, but, more particularly, because of the greatly increased incidence of bronchogenic carcinoma in later years. For instance, there were 2 cases of bronchogenic carcinoma in the first 1000 necropsies; there were 42 cases in the fourteenth 1000.

Statistical Data

Distribution by Age and Sex.—Before one delves into the more complex questions of cell type and metastasis, an otherwise undivided presentation of the distribution of the usable cases by age groups and sex is needed. This is done in Table 1. The unweighted peak of incidence for men oc-

TABLE 1.—*Distribution of 295 Necropsied Cases of Bronchogenic Carcinoma According to Age Group and Sex*

Age Group	Both Sexes		Men		Women	
	No.	Per Cent of 295* Cases	No.	Per Cent of 253 Cases	No.	Per Cent of 42 Cases
20-29	5	1.7	2	0.8	3	7.2
30-39	16	5.4	12	4.7	4	9.5
40-49	72	24.4	61	24.1	11	26.2
50-59	108	36.6	95	37.5	13	30.9
60-69	78	26.5	73	28.9	5	11.9
70-79	16	5.4	10	4.0	6	14.3
Totals	295*	100.0	253	100.0	42	100.0

* The figure is 295 instead of 296 because the exact age could not be determined for one otherwise acceptable case. This was a male patient whose neoplasm was of the adenocarcinoma type.

curred in the age period of 50 to 59 years, with 37.5% of all cases for that sex. No attempt was made to compare figures adjusted for the declining number at risk, but an incidence of 28.9% for the decade of 60 to 69 years probably does not indicate a decrease. The less sharp peak for women also was found in the age period of 50 to 59 years.

For the 296 cases, the ratio of men to women was 6.05:1. There is no reason for believing that there was any marked inequality in the number at risk for each sex; neither is there any known reason why men patients with bronchogenic carcinoma would be more prone than women patients to come to the University Hospital. This ratio, well within the range of general experience,[1] emphasizes the importance of maleness in the causation and epidemiology of this disease.

The small number (42) of female patients with bronchogenic carcinoma results in figures which are suggestive, but not necessarily reliable. However, in accordance with the experience of others,[1] there were an earlier rise, a less sharp peak in the age group of 40 to 69 years, and a somewhat higher incidence in the eighth decade of life than was found for male patients.

Distribution as to Cell Type and Sex.—In earlier papers[2,3] from this department, cases of bronchogenic carcinoma were distributed into three groups according to cell type: squamous-cell, gland-cell, and undifferentiated-cell. It was recognized that these terms were applicable with precision only when the respective types were fully exemplified, and that the less obvious examples could be assigned to one of the three groups with decreasing assurance as critical characteristics were less and less clearly manifested. Accordingly, and on the basis of cellular structure alone, it was considered useful to think of bronchogenic carcinomas as distributed on a Y-shaped figure, with well-differentiated adenocarcinomas at the extremity of one branch of the Y, cornifying squamous-cell carcinomas in a similar position on the other branch, and small, round-cell, sarcoma-like carcinomas at the lower extremity of the stem. This left the region where the three branches converged and united for examples which were of uncertain placement.

This schema was, and still is, a useful one for a well-reasoned arrangement on a morphological basis. However, some authors assigned a dynamic significance to the concept beyond that which was intended, and assumed that it meant that by differentiation or dedifferentiation any particular neoplasm must be derived from one below or above it on the Y. While it may be true that bronchogenic carcinomas of all types are derived from basal cells (in the sense of reserve cells) of the airway, the schema was intended to emphasize morphological gradations rather than derivation.

In order to make the present statistical material as useful as possible, four cell types have been recognized. This is in accord with most recent authors and with the classification used in exfoliative cytological diagnosis.[4] The squamous-cell type is continued. The designation adenocarcinoma has been accepted for the older-gland-cell group, although actual glandular formation should not be considered essential. The present undifferentiated-cell group is the undifferentiated small-cell group of the cytologists; and our poorly differentiated-cell group is that indeterminate complex group, now frequently referred to as the undifferentiated large-cell type.

TABLE 2.—*Distribution of 296 Cases of Bronchogenic Carcinoma as to Cell Type and Sex*

Type	Both Sexes		Men		Women	
	No.	Per Cent	No.	Per Cent	No.	Per Cent
Squamous-cell	107	36.15	104	40.94	3	7.14
Adenocarcinoma	106	35.81	78	30.71	28	66.67
Poorly differentiated-cell	28	9.46	26	10.24	2	4.76
Undifferentiated-cell	55	18.58	46	18.11	9	21.43
Totals	296	100.00	254	100.00	42	100.00

Not all portions of a neoplasm have the same structural features. The more numerous the areas examined, the greater is the possibility of precise classification. If wider sampling results in change in classification, it will usually be transferral from the poorly differentiated (undifferentiated large-cell) group to either the squamous-cell or the adenocarcinoma category.

In Table 2 the 296 cases are distributed according to cell type, without and with division according to sex. As is indicated by the totals, the percentages pertain to the total number in each category as to sex. In the group as a whole, squamous-cell cancers and adenocarcinomas occurred in almost equal numbers, with about one-fourth as many cases of poorly differentiated type and one-half as many of undifferentiated-cell carcinoma as of each of the other two. Thus the four cellular types were represented, in the order named, approximately in the proportion 4:4:1:2.

When distribution was according to sex, a striking and probably etiologically important difference at once became evident. Squamous-cell bronchogenic carcinoma was a male attribute to a marked degree, with 104 of 107 cases of that type occurring in men. Contrariwise, while adenocarcinoma was found in both sexes, it was the "lung cancer of women." Of the 42 bronchogenic carcinomas in women, 28, or 66.67%, were of this type.

In Table 3 the numbers of cases of each cell type are repeated, but percentages are computed for distribution between the sexes of the cases of each cell type. Only 2.80% of 107 squamous-cell cancers occurred in women. This can be compared with 26.42% of the adenocarcinomas.

The poorly differentiated-cell cancers resembled the squamous-cell type in distribution according to sex, but with less extreme dichotomy. Of all male cases, 10.24% were of this type, as were 4.76% of all female cases. When the 28 cases of this type were divided according to sex (Table 3), 92.86% were in men and 7.14% in women. This approximation to the percentages for squamous-cell cancer suggests that a considerable proportion of this undeterminable group actually may belong to the squamous-cell series.

The undifferentiated-cell carcinomas were five times as numerous in men as in women, a whole. They comprised 18.11% of the male and 21.43% of the female bronchogenic a ratio close to that (6.05:1) of the group as cancers.

Distribution of 296 Cases of Bronchogenic Carcinoma According to Age Group, Cell Type, and Sex.—When the 296 cases of bronchogenic carcinoma were tabulated ac-

TABLE 3.—*Percentage Distribution as to Sex Within Each Cell Type of 296 Cases of Bronchogenic Carcinoma*

Type	Total of Type	Men		Women		Total Per Cent
		No.	Per Cent	No.	Per Cent	
Squamous-cell	107	104	97.20	3	2.80	100
Adenocarcinoma	106	78	73.58	28	26.42	100
Poorly differentiated-cell	28	26	92.86	2	7.14	100
Undifferentiated-cell	55	46	83.64	9	16.36	100
All types	296	254	85.81	42	14.19	100

TABLE 4.—*Distribution of 296 Cases of Bronchogenic Carcinoma According to Age Group, Cell Type, and Sex*

Cell Type	Age Group	Both Sexes	Men	Women
Squamous-cell	20-29	1	1	0
	30-39	4	4	0
	40-49	20	20	0
	50-59	43	42	1
	60-69	32	32	0
	70-79	7	5	2
Adenocarcinoma	20-29	2	1	1
	30-39	8	4	4
	40-49	30	21	9
	50-59	35	25	10
	60-69	28	25	3
	70-79	2	1	1
	Unknown	1	1	0
Poorly differentiated-cell	20-29	0	0	0
	30-39	3	3	0
	40-49	9	9	0
	50-59	8	7	1
	60-69	6	5	1
	70-79	2	2	0
Undifferentiated-cell	20-29	2	0	2
	30-39	1	1	0
	40-49	13	11	2
	50-59	22	21	1
	60-69	12	11	1
	70-79	5	2	3
Totals		296	254	42

cording to age groups, as well as by cell type and sex, the resulting classes for the limited group of female cases became too small to have statistical significance. For this reason, percentages are not included in Table 4, in which the actual number of cases is set forth so that others can use these figures in compiling a larger series which may yield statistically significant data for women with bronchogenic carcinoma. Attention is called, however, to the concentration of 26 cases of adenocarcinoma in women between 30 and 69 years old, when the cases of all types and at all ages in women numbered but 42.

Patterns of Metastasis

There are practical, as well as theoretical, advantages to be derived from knowledge of the extent to which bronchogenic carcinoma metastasizes, of the usual sites of metastasis, and of differences in the patterns of metastasis associated with each of the major cell types. However, no matter how well grounded such knowledge may be, it must be remembered that it concerns the mode or trend of behavior of a neoplastic group, and that the first or any other example may behave in a manner quite at variance with the group as a whole.

It is thought that the inherent proclivity of a neoplasm to metastasize depends upon such general factors as potentiality for cell division, mode of growth in respect to invasion of blood and lymph channels, presence and character of the supporting stroma, and the level of antagonism between the neoplasm and the more normal structures. It might be expected, therefore, that differences in cell type among the bronchogenic cancers would result in different patterns of metastasis. However, metastasis is influenced also by such more or less fortuitous factors as location in respect to vascular channels, changes in blood and lymph flow, local inflammatory processes or physical trauma, and sometimes by ineffectual therapeutic attempts. Thus the clinical impression may be that chance, rather than cell type, has determined metastasis.

For statistical investigation of the frequency and sites of metastasis, it is necessary to depend upon necropsy protocols and review of stored sections. The results always will understate the truth. The most "complete" necropsy is actually much less than complete. In otherwise "complete" necropsies, certain regions may have been restricted by legal considerations. The prosector may have missed small metastases in large organs. The protocol itself may be inadequate. However, microscopical examination of sections from well-selected blocks, including bone marrow, will reveal some metastases which could not have been found by gross examination alone. The greater the number of sections examined, the closer will be the approach to the truth.

The necropsy material upon which this study was based included an average of 70 sections from each case. Even so, it is realized that the reported incidence of metastasis is less than the true incidence to an unknown degree.

It might be considered that for a comparative study of the patterns of metastasis from primary bronchogenic cancers of the

TABLE 5.—*Distribution as to Cell Type of Thirty-One Cases of Bronchogenic Carcinoma in Which No Metastases Were Found at Necropsy*

Cell Type	Ratio*	Percentage
Squamous-cell	23/107	21.50
Adenocarcinoma	4/105	3.77
Poorly differentiated-cell	1/28	3.57
Undifferentiated-cell	3/55	5.45
All types	31/296	10.47

* Expressed as a fraction of which the numerator is the number of cases without metastases and the denominator the number of cases of that cell type.

four cell types, the burden of incomplete discovery would weigh equally upon each type, and therefore could be disregarded. However, this is not entirely true. To some degree, metastasis is a function of duration of the disease. Squamous-cell bronchogenic carcinoma usually gives rise to symptoms at a much earlier stage than neoplasms of the other three types. This is due to the fact that it tends to produce stenosis of a bronchial orifice while the primary is yet small. The signs and symptoms lead to early diagnosis, and therefore to lobectomy or pneumonectomy. In the series under review, thoracotomy was performed on 87 patients and 69% of these had squamous-cell carcinomas. Of this 69%, some died on the operating table, some died postoperatively from pulmonary embolism or hemorrhage, and some recovered, but died later for reasons quite apart from carcinoma of the lung, and with no residual neoplasm. Thus the series tends to be unduly weighted with cases of squamous-cell carcinoma of shorter duration (in respect to total span of the disease, rather than to calendar time) than that of most cases of the other three types. This must be kept in mind in the statistical analysis which follows. There is need for a comprehensive study of the metastatic pattern of squamous-cell carcinoma based only on cases which were beyond the resectable stage when the diagnosis was made.

In 31 of the 296 necropsied cases of bronchogenic carcinoma under analysis no metastases were found. In Table 5 these are distributed according to cell type, and the actual numbers of cases and the percentage for each group are shown. Squamous-cell carcinomas alone were found to have a significant representation (21.50%) of cases without discoverable metastases. The other three types showed no important difference among themselves in the remarkably small number (8/189, or 4.23%) of cases without metastases terminally.

In order to obtain as broad an initial picture as possible, the 265 cases with recognized metastases were distributed as to cell type and the occurrence of secondary neoplasms in four target areas: nervous system, thoracic region, abdominal region, and skeleton. It will be noted that these are four regions in which the development of metastatic bronchogenic cancer may determine the presenting signs and symptoms. The results of this distribution are shown in Table 6, in which correction has been made for those occasional cases in which particular target areas could not be examined.

Apparently significant similarities and differences among the four cell types appear in Table 6. Each of the four target areas was vulnerable to every cell type, but to varying degrees. Every cell type showed its highest percentage of metastases, or extension(?), in the thoracic region. Squamous-cell bronchogenic carcinoma, perhaps for the reasons developed previously, with 76.64% of thoracic metastases, was unlike the other types, all of which showed percentage values

TABLE 6.—*Occurrence and Distribution of Metastases as to Cell Type of the Primary Neoplasms and Target Areas*

Target Area	Cell Type	Ratio*	Percentage
Nervous system	Squamous	15/73	20.55
	Adenocarcinoma	38/84	45.24
	Poorly differentiated	4/20	20.00
	Undifferentiated	11/39	28.21
Thoracic region	Squamous	82/107	76.64
	Adenocarcinoma	98/106	92.45
	Poorly differentiated	27/28	96.43
	Undifferentiated	51/55	92.73
Abdominal region	Squamous	46/104	44.23
	Adenocarcinoma	70/103	67.96
	Poorly differentiated	18/28	64.29
	Undifferentiated	41/54	75.93
Skeleton	Squamous	15/107	14.02
	Adenocarcinoma	40/105	38.10
	Poorly differentiated	12/26	46.15
	Undifferentiated	19/55	34.55

* The numerator is the number of cases of the specified cell type with metastases to the target area. The denominator is the total number of cases of that cell type minus those for which permission to examine the target area was withheld.

well above 90. From the practical standpoint this means that thoracic spread must be expected before death in practically all cases of bronchogenic carcinoma of adenocarcinomatous, poorly differentiated-, or undifferentiated-cell types, and in three-fourths of those of squamous-cell type. This high rate of metastasis in proximity to the primary site must be brought about largely by lymphogenous spread. There may be some error in these high figures because of the impossibility of differentiating in all cases between spread by continuity and true metastasis.

All types produced metastases to the central nervous system, usually to the brain, but the adenocarcinomatous group showed twice as great a proclivity (45.24%) in this respect as squamous-cell cancer. The undifferentiated type was in second place. The results for the poorly differentiated type may not be valid because of the small numbers for this group.

Undifferentiated-cell carcinoma showed the highest incidence of metastases to abdominal structures and squamous-cell carcinoma the lowest. The difference between adenocarcinoma and squamous-cell carcinoma (67.96% *vs.* 44.23%) gains in significance in view of the relatively large groups of each type, differing by only one case in total number.

Having in mind the very small portion of the skeletal system which can be examined grossly or sampled microscopically, it is dismaying to find that in from 14.02% (squamous-cell) to 46.15% (poorly differentiated-cell) of the cases of bronchogenic-carcinoma metastases to bone were discovered. Adenocarcinoma was almost three times (38.10%) as prone to give skeletal metastases as squamous-cell carcinoma. Skeletal metastases must be almost entirely due to hematogenous dissemination.

There has been special interest in certain organs as target areas for metastasis from bronchogenic carcinoma. The extent of involvement of these areas in actual numbers and as percentages is shown in Table 7. The corresponding figures for the central nervous system and skeleton are repeated from Table 6 for comparison.

In the thorax, the regional lymph nodes were extensively involved by cancers of all cell types, ranging from 92.86%, for the poorly differentiated form, to 64.49% for squamous-cell carcinomas. High values were found for lung for all cell types. Unfortunately, distinction could not be made between the lung of the primary lesion and the contralateral organ. This distinction was usually available in the gross descriptions, but the figures for lung included numerous cases in which metastases were found microscopically, and from which lung particular

TABLE 7.—*Distribution of 296 Cases of Bronchogenic Carcinoma as to Cell Type and Metastasis to Specified Organs as Target Areas*

	Squamous-Cell		Adenocarcinoma		Poorly Differentiated-cell		Undifferentiated-cell	
Target Organ	Ratio*	Percentage	Ratio *	Percentage	Ratio*	Percentage	Ratio*	Percentage
Central nervous system	15/73	20.55	38/84	**45.24**†	4/20	20.00	11/39	28.21
Skeleton	15/107	14.02	40/105	**38.10**	12/26	**46.15**	19/55	**34.55**
Thoracic region								
Heart	9/107	8.41	18/106	16.98	3/28	10.71	9/55	16.36
Regional nodes	69/107	**64.49**	86/106	**81.13**	26/28	**92.86**	46/55	**83.64**
Lung	59/107	55.14	72/106	67.92	24/28	85.71	39/55	70.91
Thyroid	6/107	5.61	7/106	6.60	2/28	7.14	5/55	9.09
Abdominal region								
Lymph nodes	25/104	**24.04**	42/103	**40.78**	16/28	**57.14**	19/54	**35.19**
Liver	32/104	**30.77**	39/103	**37.86**	12/28	**42.86**	23/54	**42.59**
Kidney	15/104	14.42	26/103	**25.24**	3/28	10.71	13/54	**24.07**
Spleen	5/104	4.81	12/103	**11.65**	1/28	3.57	4/54	7.41
Adrenal glands	19/104	18.27	44/103	**42.72**	9/28	**32.14**	20/54	**37.04**
Pancreas	3/104	2.88	11/103	**10.89**	5/28	**17.86**	9/54	**16.67**

* The numerator is the number of cases of the specified cell type with metastases to the target organ. The denominator is the total number of cases of that cell type minus those for which permission to examine the target organ was withheld.
† Percentages of special clinicopathological significance are shown in boldface type.

sections were taken was seldom indicated. Differentiation between extension by continuity and discontinuous lymphogenous metastasis may be difficult or impossible in the lung of origin; just as it may be in mediastinal structures.

It will be noted that there were 20 cases with metastases in the thyroid gland. All cell types were represented, without great difference among them percentage-wise, but the small numbers impair the significance of the comparative distribution. Undifferentiated-cell carcinomas showed the highest percentage and squamous-cell carcinomas the lowest. When the thyroid was extensively involved by other than squamous-cell cancer, there were occasional cases which gave rise to a reasonable doubt whether the primary was in the bronchus or the thyroid.

The abdominal lymph nodes showed a comparatively high incidence of metastatic involvement, the range being from 24.04%, for the squamous-cell type, to 57.14%, for the poorly differentiated-cell type. Neoplasms of all types metastasize freely to the liver. Squamous-cell carcinoma had the lowest percentage, but even for it the incidence was 30.77%.

The usual experience in respect to metastasis to the adrenal glands was reflected in this series. All types gave many examples, for a total of 92 cases. Adenocarcinoma showed more than twice the propensity of squamous-cell carcinoma to disseminate to this site, the respective percentages being 42.72 and 18.27. Poorly differentiated- and undifferentiated-cell types were only slightly less prone than adenocarcinoma to metastasize to the adrenal glands.

Adenocarcinoma and undifferentiated-cell carcinoma gave the more frequent renal metastases. All types except squamous-cell cancer produced pancreatic metastases in from 10% to 20% of the cases of the respective types.

The marked tendency of adenocarcinoma to produce hematogenous metastases was shown for all abdominal target organs tabulated. Even the spleens had metastases in 11.65% of the cases of adenocarcinoma. Undifferentiated-cell carcinoma was in second place, with 7.41%. Further distribution of the material as to sex, age group, cell type, and metastasis, considered simultaneously, gives groups of which many are too small to have significance. Naturally, the prevalence of squamous-cell carcinoma in men and the dominance of adenocarcinoma in women are reflected in the results. This tabulation will not be presented in full, but the numerical data for the female patients is given in Table 8 for whatever value it may have for future compilations by others.

Association Coefficients for Pattern of Metastasis of Bronchogenic Carcinoma

The percentage of cases of each of the four cell types which had produced metastases to particular sites is shown in Table 7. This does not give a complete concept of the comparative tendency of metastasis to occur in relation to all cases of bronchogenic carcinoma, because the per-

TABLE 8.—*Number of Cases of Bronchogenic Carcinoma in Female Patients with Metastasis to Major Target Areas, by Age Groups and Cell Types*

Age Groups, Yr.	Squamous-Cell (3)*				Adenocarcinoma (28)				Poorly Differentiated-Cell (2)				Undifferentiated-Cell (9)			
	CNS†	Skel.	Thor.	Abd.	CNS.	Skel.	Thor.	Abd.	CNS.	Skel.	Thor.	Abd.	CNS.	Skel.	Thor.	Abd.
20-29						1	1							1	2	2
30-39						1	2	3								
40-49					5	5	7	7							1	
50-59		1	1	1	6	3	8	7		1	1					
60-69					1		3	1		1	1	1	1		1	1
70-79			2											1	2	2

* The numbers in parentheses give the number of cases of that cell type in the entire group of 42 cases in women. All had produced metastases except three of the nine cases of undifferentiated-cell type.

† The four major target areas considered are the central nervous system, skeleton, thoracic area as a whole, and abdominal area as a whole.

centage is not influenced by the extent to which the other three types did, or did not, produce metastases to that site. When Samson [5] investigated this question, in 1935, he made use of the method proposed by Yule [6] for deriving a relative numerical value for a fourfold association. For the derivation of the formula, the original source should be consulted.

The association coefficient Q is computed by simple arithmetical procedures from the formula

$$Q=\frac{(AB)(CD)-(AD)(BC)}{(AB)(CD)+(AD)(BC)}$$

A and B signify attribute occurrence, and C and D signify attribute nonoccurrence. Taken singly these symbols have no numerical value. But AB-attribute concurrence and BC, AD, and CD=the three possible varieties of attribute nonconcurrence. These four classes can be recognized and enumerated and thus acquire numerical values. Thus (AB)=the number of cases with metastases of a specified type at the specified site; (BC), the combined numbers of cases of the other three cell types with metastases to the specified site; (AD), the number of cases of the specified cell type without metastases to the specified site, and (CD), the combined numbers of cases of the other three cell types without metastases to the specified site.

By referring to Table 7, for example, it will be found that for squamous-cell bronchogenic carcinoma and metastasis to the central nervous system

$$Q=\frac{(15)(46+16+28)-(58)(38+4+11)}{(15)(46+16+28)+(58)(38+4+11)}=-0.390$$

Values for Q will always fall in the range of -1.0 through 0.0 to $+1.0$. These figures are not percentages or ratios but do indicate the strength of association between a particular type of bronchogenic carcinoma and the occurrence of metastases at a particular site. Furthermore, this association is not one of comparison within the type. That relationship is indicated by the actual percentage, as shown in Table 7. The association coefficient Q is influenced equally by the behavior of carcinomas of all other types. Thus the coefficient -0.390 is an expression not only of the tendency of squamous-cell bronchogenic carcinoma to produce metastases to the central nervous system, but also of the tendency of all other bronchogenic carcinomas to produce, or not to produce, metastases to that target area. A value for Q of 0.00 would indicate complete independence of the two pairs of attributes compared: cell type occurrence and nonoccurrence, with specific target area occurrence and nonoccurrence. A value of $Q=-1.0$ would indicate that with the specific cell type the specific metastasis never occurs. A value of $Q=+1.0$ would indicate that with the specific cell type the specific metastasis always occurs.

Association coefficients for selected targets are shown in Table 9. These values are true only for the group of 296 cases under analysis. They would differ slightly with each new case added to the series, but the group was sufficiently numerous to suggest that the coefficients presented are representative and that general conclusions can be drawn from them. When they are compared with similar coefficients derived by Samson [5] from a group of 100 cases in 1935, similar trends can be recognized, but there are also marked differences, reasons for which are not entirely clear. They are not due to the fact that Samson's cases included 51% of adenocarcinomas. The value of Q is not altered by changing the proportionate numbers of cases of each type, provided the attributes of the types remain the same. That Samson used but three categories as to cell type does not offer an adequate explanation. Probably the most important factor has been the increased use of radical thoracic surgery at a time when squamous-cell bronchogenic carcinoma is becoming increasingly commoner. This tends to give undue weight in a necropsy series to operative and postoperative deaths of patients with squamous-cell cancer whose disease, because of the earlier diagnosis of this type, has not reached the stage of widespread metastasis.

The association coefficients of Table 9 are remarkably consistent. Negative values were found for every target organ in respect

TABLE 9.—*Association Coefficients* Indicating the Tendency of a Specific Cell Type of Bronchogenic Carcinoma to Metastasize to a Specific Target, Compared with the Tendency of All Other Types to, or Not to, Metastasize to That Target*

Target Organ	Cell Type			
	Squamous	Adenocarcinoma	Poorly Differentiated	Undifferentiated
Central nervous system	−0.390	+0.475	−0.320	−0.095
Skeleton	−0.582	+0.310	+0.382	+0.148
Thoracic lymph nodes	−0.475	+0.198	+0.625	+0.258
Liver	−0.200	+0.040	+0.143	+0.152
Pancreas	−0.681	+0.086	+0.385	+0.389
Adrenal glands	−0.489	+0.364	+0.008	+0.142
Kidneys	−0.271	+0.256	−0.370	+0.158
Spleen	−0.334	+0.398	−0.405	−0.018

* Yule.[4]

to squamous-cell carcinoma and positive values for each of the same sites with adenocarcinoma. Most of these sites presumably would be reached only by hematogenous dissemination. The proclivity of bronchogenic adenocarcinoma in this respect is demonstrated emphatically.

Poorly differentiated cancer presented a mixed picture, with several values close to the ±0.0 range. Only for thoracic lymph nodes was there a strong positive association. This suggests that many of the cancers of this type were actually poorly differentiated squamous-cell neoplasms.

The undifferentiated-cell carcinomas behave more nearly like adenocarcinoma. The only two negative values, −0.095 for central nervous system and −0.018 for spleen, are but very little below the 0.0 level.

To avoid misinterpretation, attention is called again to the fact that the association coefficients presented in this section cannot be used as precentages. Metastasis of squamous-cell bronchogenic carcinoma to the central nervous system can be taken as an example. From Table 7 it can be ascertained that 15 (20.55%) of the 73 squamous-cell carcinomas had metastasized to the central nervous system. This is almost exactly one-fifth of the group. Yet when the concurrence and nonconcurrence of the attributes of squamous-cell and of central nervous system metastasis were subjected to fourfold analysis in connection with similar attribute concurrence and nonconcurrence for the other three cell types in the total population of 296 cases, a strongly negative association coefficient (−0.390) was found. This is explained by the far greater tendency of adenocarcinoma to produce such metastases (45.24%; association coefficient, +0.475).

Summary

A statistical analysis has been made of 296 cases of bronchogenic carcinoma from the necropsy files of the University of Michigan.

There were 254 cases in men and 42 in women, giving a ratio of 6.05:1.

The largest number of cases for each sex was found in the age period of 50 to 59 years. (No attempt was made to adjust age distribution for declining numbers at risk in subsequent decades.) Percentage-wise, for women there was an earlier rise, a less sharp peak in the age group of 40 to 69 years, and a somewhat higher incidence in the eighth decade of life than for men.

The 296 cases were divided into four groups as to cell type: squamous-cell carcinoma, adenocarcinoma, poorly differentiated-cell carcinoma, and undifferentiated-cell carcinoma. These were represented, in the order named, approximately in the proportion 4:4:1:2.

Distribution according to cell type and sex emphasized the degree to which squamous-cell bronchogenic carcinoma is a male attribute. Of 107 squamous-cell cancers, 104 (97.20%) were found in men. The reciprocal effect, i. e., the dearth of cancers of this type among women, gives added weight to adenocarcinoma in the distribu-

tion for that sex. Of the 42 bronchogenic cancers in female patients, 28 (66.67%) were adenocarcinomas. The poorly differentiated-cell cancers resembled the squamous-cell type in distribution as to sex. Undifferentiated-cell carcinomas were divided between men and women in a proportion approximately that of the entire group.

The data for bronchogenic carcinoma in women do not have the same degree of significance as those for men because of the relatively small number of cases.

The patterns of metastasis for each cell type have been investigated by both percentages and association coefficients. In 31 of the 296 necropsies metastatic neoplasm was not found, and 23 of these were of squamous-cell type.

All target areas investigated were vulnerable to metastasis from carcinomas of all four cell types, but to different degrees. The thoracic area showed the highest percentages for all types.

All methods of analysis indicated the marked proclivity of bronchogenic adenocarcinoma to produce hematogenous metastasis, with poorly differentiated- and undifferentiated-cell neoplasms only slightly less active in this respect. In a fourfold evaluation of the attributes of the combined groups, negative association coefficients were found for squamous-cell cancer and positive coefficients for adenocarcinoma for metastasis to every target area investigated.

The behavior of the poorly differentiated-cell bronchogenic carcinomas is such as to suggest that many in this group are closely related to squamous-cell carcinoma.

The lower percentage values and negative association coefficients for squamous-cell bronchogenic carcinomas may have been influenced by the earlier diagnosis and more frequent radical surgical treatment of this form. There is need for a special study of metastasis of squamous-cell cancer in cases in which the neoplasm has completed its course without surgical intervention.

The results of this analysis are in general agreement with those of others. It was considered appropriate to make the basic data available for the compilation of larger collections of the less common forms, such as squamous-cell bronchogenic carcinoma in women.

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Role of Mural Fibrin Thrombi of the Aorta in Genesis of Arteriosclerotic Plaques

Report of Two Cases

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The purpose of this paper is to show that lesions appearing grossly either as white fibrous arteriosclerotic plaques or as caps covering atheromata may originate from mural fibrin thrombi.

Two cases of our recent autopsy material were outstanding examples of an intima studded with numerous white plaques that proved on microscopic examination to be mural fibrin thrombi in various stages of organization.

CASE 1.—*Clinical Summary.*—Woman, age 40. This patient was admitted to hospital on Sept. 26, 1955, having had previous admissions, for which the old records were not available. In November, 1954, a diagnosis of chronic glomerulonephritis with azotemia was made, and her blood urea nitrogen was 45.5 mg. per 100 cc. On this admission she was unable to give a clear history but had noted a gradual decrease in exercise tolerance, with orthopnea and episodes of nocturnal dyspnea of one week's duration. She also complained of pruritus of 7 to 10 days' duration, nocturia (five to six times), and polyuria. On examination her tongue was dry and coated and she had a uremic odor. She was dyspneic with orthopnea and a respiratory rate of 30 a minute. There was minimal cyanosis of her lips, and her skin was very excoriated from scratching, with areas of blotchy ecchymoses and many petechiae. The skin was described as having a dusky hue, with a cafe au lait appearance. There was moderate cervical venous distention. The blood pressure was 230/128. The pulse rate was 110, with a Grade 2 apical systolic murmur and a protodiastolic gallop at the apex. A coarse friction rub was heard over the entire precordium. The chest was clear to percussion, but there were many fine crepitations heard throughout the entire lung fields. Examination of the fundi showed marked retinopathy with many recent and old hemorrhages present. The patient was given a salt-free diet, intravenous fluids, hydralazine (Apresoline), digitalis, and morphine and was placed in an oxygen tent. Her condition deteriorated, with drowsiness and restlessness, and she died three days after admission.

Laboratory Findings: Hemoglobin 6.7 gm. per 100 cc.; RBC 2,230,000; WBC 18,000; sedimentation rate 135 mm. in one hour; hematocrit 23%; blood urea nitrogen 123.1 mg., serum cholesterol 211 mg., serum sodium 117 mEq/liter, serum potassium 7.2 mEq/liter, serum chloride 94.2 mEq/liter, per 100 cc.

Anatomical Diagnoses.—Chronic pyelonephritis and malignant nephrosclerosis: (1) chronic and acute pyelonephritis; (2) malignant nephrosclerosis; (3) uremia (BUN 123.1 mg. per 100 cc.); (4) hypertension (B. P. 230/128); (5) dilatation and hypertrophy of heart (wt. 500 gm., expected normal 288 gm.); (6) multiple small infarcts of myocardium; (7) arteriolar necrosis of kidney, spleen, pancreas, and heart; (8) acute fibrinous pericarditis, uremic; (9) bronchopneumonia (uremic); (10) edema of lungs; (11) pleural effusion (right, 250 cc.); (12) fibrinous pleural adhesions, left; (13) arteriosclerosis of aorta, and coronary, renal, and inferior mesenteric arteries; (14) chronic passive congestion of liver; (15) nodular colloid goiter; (16) acute cystitis; (17) digitalis therapy.

Gross and Microscopic Findings of Aorta.

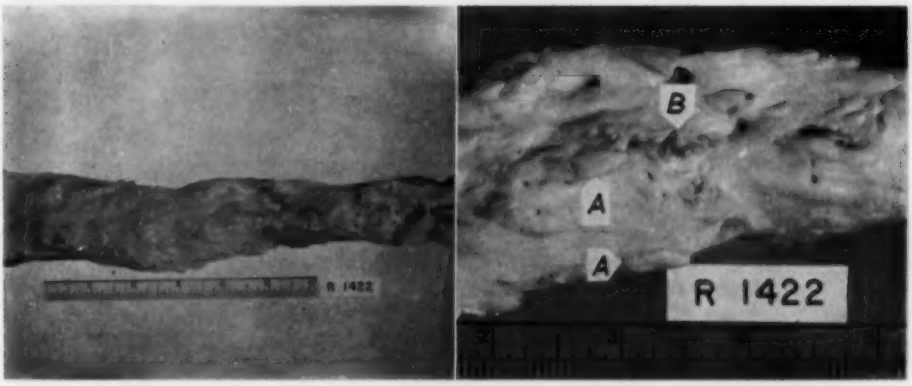
Numerous large, pearly-white plaques were distributed throughout the aorta (Figs. 1 and 2). They were most numerous along the intercostal and lumbar arteries. Superimposed on these, as well as on yellow,

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Figs. 1 and 2.—Gross appearance of aorta of Case 1. Numerous fibrous plaques are present throughout the aorta. They have a polypoid appearance (*A*). A few yellow flecks and streaks, as well as numerous large yellow plaques, ulcerated in some areas (*B*), are also seen.

plaques were white opaque plaques, measuring 0.3-2 cm. in length and 0.3-0.5 cm. in width. These last structures often had a polypoid appearance. A few yellow flecks and streaks and numerous large yellow plaques contributed to the pleomorphism of the aortic surface. Some of these plaques were ulcerated. A slight red tinge was noticeable in some of the yellow, as well as in the white, plaques. The orifices of the inferior mesenteric and the left renal artery were partly occluded by the above-described pearly-white plaques, which were superimposed on yellow atheromatous areas within the arterial wall. The aorta between these focal lesions showed more or less diffuse longitudinal wrinkling.

Numerous sections were stained by various staining methods. These methods have been described in detail in another paper.[5]

Microscopically, the white opaque plaques consisted of recent thrombi composed mainly of fibrin. These were deposited on pearly-white plaques, yellow plaques, or yellow ulcerated areas. The pearly-white plaques were composed either entirely of a young, acid-mucopolysaccharide (AMP)-rich connective tissue or of AMP-rich tissue in which remnants of unorganized thrombus material were still present. Lipid-containing lesions showed intense diffuse intracellular deposition of lipid with little or marked atheromatous disintegration. These fatty lesions were covered usually by a thick layer of AMP-rich tissue, which, in turn, was often covered by recent thrombus material.

Upon cross section, certain lesions appeared grossly as flat yellow bands in the intimal region, covered by two layers of white material with some red streaking on their surface; microscopically they were characterized as follows: At low power (Fig. 3) there was above the media a layer of approximately the same thickness as the media composed of collagen, ground substance, and elastic fibers. Intra- and extracellular lipid material and young, organizing granulation tissue were present in the more superficial parts. The latter also contained remnants of unorganized thrombus material, more abundant toward the lumen. A recent thrombus was composed mainly of a dense meshwork of fibrin (Fig. 4). Numerous cells and platelets were entrapped in the network of fibrin.

The layer underlying the recent thrombus was composed of a thrombus which was organized, more so in the depth than superficially. The unorganized thrombus material appeared as a homogeneous acidophilic material in varying-sized clumps, streaks, and masses, which in the slide stained with Alcian blue-PAS-orange G appeared as a deep orange. The organized parts consisted

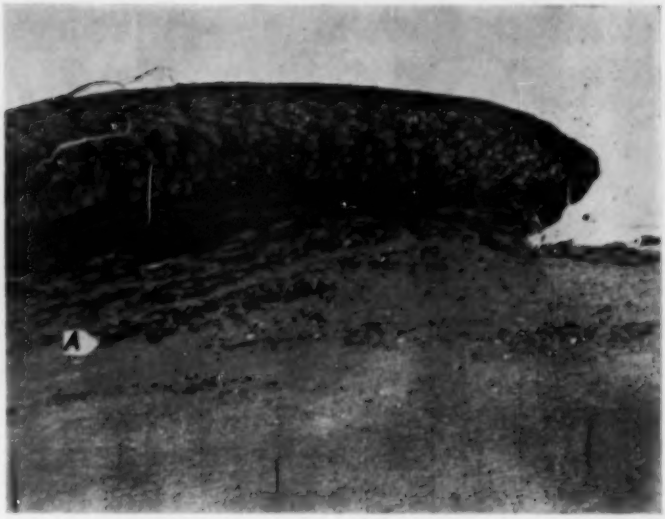


Fig. 3.—Low-power view of plaques seen in Figures 1 and 2. A recent thrombus is seen on the surface of the aorta and a partly organized thrombus between the recent one and the media. Unorganized remnants of the homogeneous fibrinoid-like thrombus material are seen in dark gray (*A*). Pentachrome I (elastica omitted); reduced to 73% of mag. × 54.

of a young connective tissue. This was composed of a homogeneous AMP-containing faint-blue material and delicate purplish-red fibers, which were condensed around newly formed capillaries (Fig. 5). In Pentachrome II the newly formed ground substance stained blue; the delicate and coarser collagen fibers arising in the ground substance were red, and the unor-

Fig. 4.—High-power view of recent thrombus seen in Figure 3. A dense meshwork of black-staining fibrin is seen in which numerous cells are entrapped. PTAH; reduced to 89% of mag. × 615.

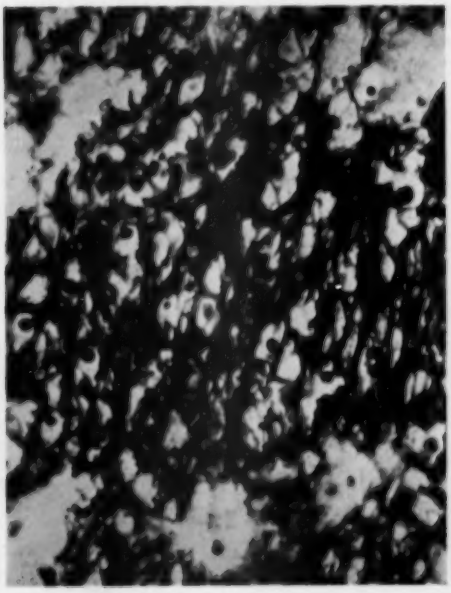


Fig. 5.—Area from partly organized thrombus material. Three new capillaries have been formed (*A, B, C*). Their PAS-positive basement membranes are seen in dark gray; the blue-staining ground substance in pale gray, and the unorganized thrombus material in black. Alcian blue-PAS-orange G; reduced to 89% of mag. × 207.



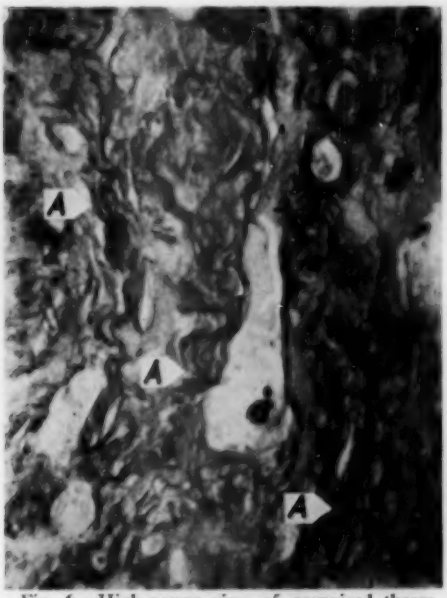


Fig. 6.—High-power view of organized thrombus material, demonstrating the newly formed delicate collagen fibers (*A*-blue in section, black in photograph). A capillary is seen in the center of the picture. Lillie's Allochrome procedure; reduced to 89% of mag. × 461.

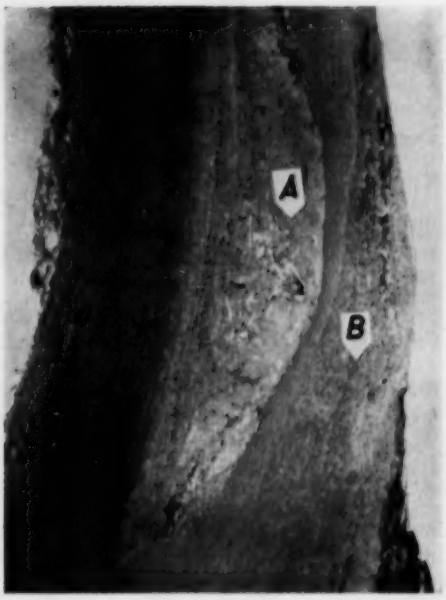
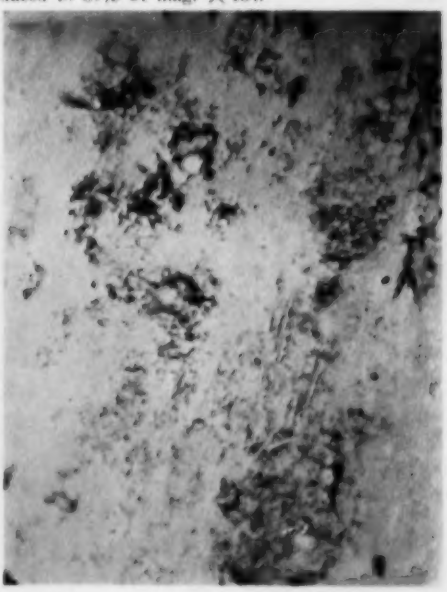


Fig. 7.—Atheroma (*A*) covered by a partly organized (*B*) thrombus, and the latter covered partly by a recent thrombus. Hemalum-phloxine-saffron; reduced to 89% of mag. × 20.

Fig. 8.—High-power view of partly organized (*B*) thrombus seen in Figure 7. The unorganized remnants of thrombus material are seen in black (*A*) and the ground substance, which contains numerous smooth muscle cells, in gray. Hemalum-phloxine-saffron; reduced to 89% of mag. × 102.



Fig. 9.—High-power view of atheroma (*A*) seen in Figure 7. The intensely acidophilic, unorganized fibrinous material is seen in black in photomicrograph. Hemalum-phloxine-saffron; reduced to 89% of mag. × 184.



ganized thrombus material was yellow. Collagen fibers seemed to be formed independently of vessels but most abundantly around the newly formed capillaries. Further separation of newly formed connective tissue elements was achieved with Lillie's Allochrome procedure, in which basement membranes of capillaries stained purplish red and the newly formed connective tissue fibers, deep blue (Fig. 6). The ground substance was unstained, or stained a very faint blue.

Extracellular lipids were present as a homogeneous band above the media, interspersed with lipophages. Some fat was present in both forms in the partly organized thrombus.

When fibrous caps were seen over atheromata, they appeared pearly white, and were, in turn, often covered by a white opaque layer of recent thrombus material (Fig. 7). The opaque layer had the microscopic appearance of the recent thrombus material described above. The pearly-white plaque over the atheroma consisted of an AMP-rich connective tissue, which often contained remnants of unorganized thrombus material (Fig. 8). Unorganized fibrinous material was often still present within the atheroma (Fig. 9).

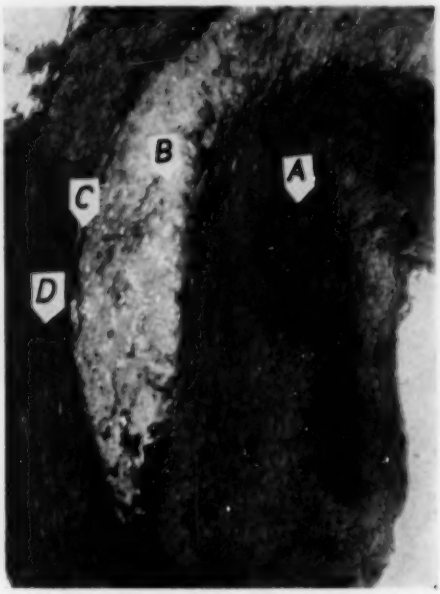
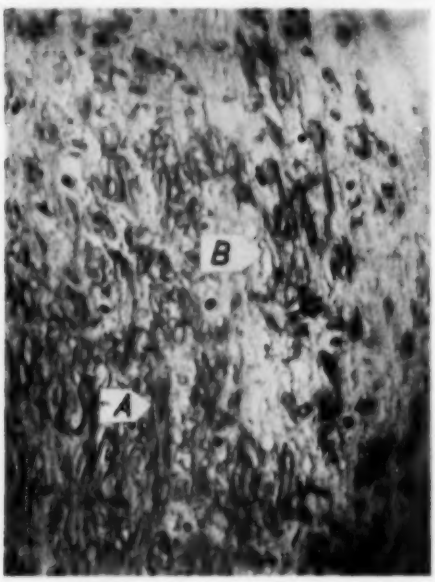


Fig. 11.—Atheroma (*B*) at the base of an organized thrombus (*A*) of the inferior mesenteric artery. The atrophic media (*D*) is seen below the internal elastic lamina (*C*). Unorganized thrombus material is seen mainly in and at the border of the atheroma. Pentachrome 1; reduced to 89% of mag. × 27.

Fig. 10.—Higher-power view of smooth muscle cells present in the organized thrombus material seen in Figures 7 and 8. The smooth muscle cells are surrounded by delicate elastic membranes (*A*), and some elastic fibers are seen also between the cells (*B*). Weigert-Hart elastica-nuclear fast red-metanil yellow; reduced to 89% of mag. × 410.



When the organization in the pearly-white plaques was fairly advanced, numerous cells were present in it. Most of these cells were not fibroblasts, as one might expect, but young, primitive smooth muscle cells which had developed in the AMP-rich matrix. These cells were surrounded by reticular and elastic fibers (Fig. 10). These smooth muscle cells were best seen in sections from the left renal and inferior mesenteric arteries. Both these vessels were obliterated in their proximal parts by organized thrombus material. An atheroma with organized thrombus material lay at the base of the organized thrombus along the internal elastic lamina (Fig. 11). Numerous young

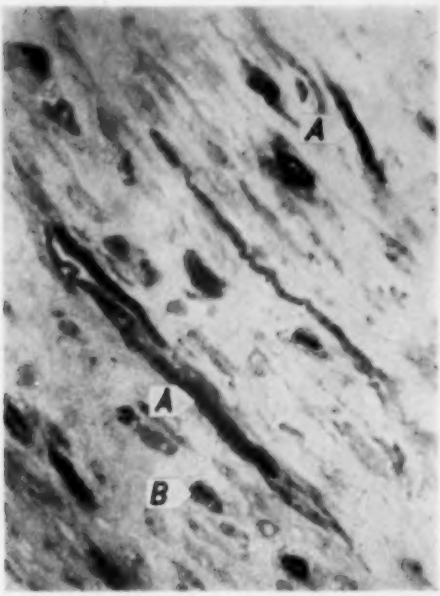


Fig. 12.—High-power view of organized thrombus seen in Figure 11, containing numerous smooth muscle cells. Some of these cells have been cut longitudinally (*A*) and some transversely (*B*). Trichrome stain; reduced to 89% of mag. × 973.

smooth muscle cells, similar to those seen in the aorta, were present in the organizing thrombi. These cells had elongated, cigar-shaped, and often wavy nuclei and evenly distributed chromatin, with no definitely demonstrable nucleoli. They had slender cytoplasms, in which frequently two or more intensely acidophilic myofibrils were demonstrated. The latter were coarser than those in normal smooth muscle cells and often appeared fused (Fig. 12). Only a few fibroblasts were present in these lesions.

Lipid material was at times demonstrable in both the recent and the organizing thrombi. In the recent thrombi small quantities were present extracellularly, and presented the appearance of being derived from the disintegration of cellular debris. More lipid was present intracellularly in lipophages. In the organizing part of the thrombus, usually in its depth, there was at times abundant intra- and extracellular lipid (Fig. 13). Most of the intracellular lipid was present in the macrophages, but some occurred also in primitive mesenchymal cells and in smooth muscle cells.

The outer two-thirds of the media contained a moderate number of capillaries. These capillaries were surrounded by a small number of lymphocytes and histiocytes. Some degree of atrophy was present, recognizable as fragmented and stretched elastic fibers, and atrophic smooth muscle cells.

Case 2.—*Clinical Summary.*—A man, age 86, was admitted on Jan. 15 to the Kingston General Hospital, 20 days before his death. The patient was thought to have had hypertension for a number of years. He had pneumonia in 1947. He was in hospital in the autumn of 1955 for removal of a cataract from the right eye. He developed a severe episode of diarrhea, lasting for four days, shortly before the present admission. The chief complaint on admission was shortness of breath and a sensation of fullness in his chest. Examination at that time revealed a rather grossly disorientated person, showing marked cachexia. Pulse rate was 120, with frequent extrasystoles. The blood pressure was 175/105. The apex beat was 4 in. (10 cm.) from the midline in the fifth intercostal space. A systolic murmur was heard in the aortic area. Numerous fine rales, particularly in the right lung field, were also heard. A

Fig. 13.—Intra- and extracellular lipids (gray to black) in organized thrombus. *Fettrot*-hematoxylin stain; reduced to 89% of mag. × 102.



diagnosis of generalized arteriosclerosis and mild congestive heart failure was made at that time.

Treatment consisted of salt-free diet, gitalin (Gitaligen) 0.5 mg., and mercaptomerin sodium U. S. P. (Thiomerin sodium) 2 cc. q. i. d., and Dalitol (ammonium chloride, 0.5 gm. [Horner's Company]).

Laboratory Findings: Hemoglobin 13.2 gm. per 100 cc.; WBC 6550; BUN 11.6 mg. per 100 cc. On Jan. 16 urinalysis was negative except for 1+ albumin. Jan. 25: BUN 41.5 mg. per 100 cc.; sedimentation rate 78 mm. in one hour. The BUN was 56.2 mg. per 100 cc. on Jan. 28. An ECG showed diffuse myocardial disease, and probably a digitalis effect. The patient deteriorated progressively, becoming more dyspneic, developing coarser chest rales, and becoming more disoriented. Cheyne-Stokes respirations began six hours before death, and he died uneventfully 20 days after admission.

Anatomical Diagnosis.—Generalized arteriosclerosis and bronchopneumonia: (1) generalized arteriosclerosis; (2) arteriosclerosis of coronary, cerebral, and peripheral arteries and aorta; (3) essential hypertension; (4) diffuse myocardial fibrosis, moderate; (5) calcification of aortic valve, moderate; (6) hypertrophy and dilatation of heart (wt. 450 gm., expected normal 305 gm.); (7) myocardial failure; (8) chronic passive congestion of lungs and liver (mild); (9) acute bronchopneumonia, bilateral; (10) fibrinous pleuritis; (11) pleural effusion (left, 500 cc.); (12) arteriolar nephrosclerosis, moderate; (13) uremia (BUN 56.2 mg. per 100 cc.); (14) thrombosis of periprostatic veins; (15) deformity of sternum; (18) emaciation, moderate; (19) senility; (20) benign hypertrophy of prostate.

Fig. 14.—Large recent thrombus (*A*) extending over a considerable area of the aorta. An atheroma (*B*) is seen at the bottom of the photomicrograph. Pentachrome II (elastica omitted); × 3. 8.



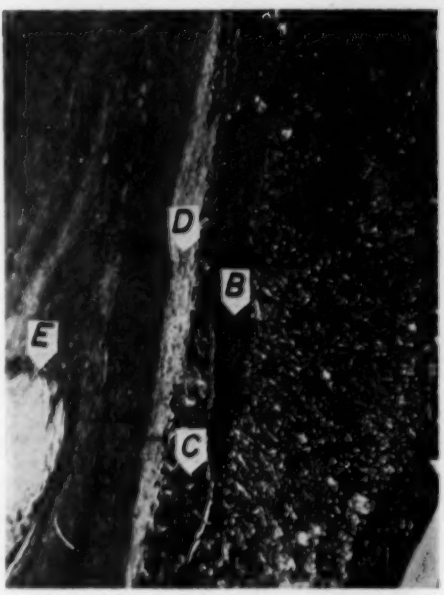


Fig. 15.—Recent (*A*) thrombus is seen above an older thrombus (*B*). The latter (black in photomicrograph, red in slide) had a fibrinoid-like appearance. A still older layer (*C*) of thrombus material was hyaline in nature (dark gray in photomicrograph and bluish red in slide). Numerous foam cells (*D*) are present between the hyaline-like substance and the diffusely thickened intima. An atheroma (*E*) is seen in the left lower corner of the photomicrograph. Trichrome stain; reduced to 89% of mag. × 30.

Gross and Microscopic Findings of Aorta.—The gross and the microscopic lesions in the second case were almost identical with those in the first case and will therefore be described briefly. Numerous large and small pearly-write and opaque white plaques were present throughout the aorta, but mainly in the abdominal part of the vessel. The lesions were less numerous but had all the characteristics of those described in the first case.

Thrombi of recent origin often attained a huge size. Some thrombi measured up to 2.5-3 cm. in length (Fig. 14). In such lesions one could often see several layers (Fig. 15). A recent thrombus composed of a meshwork of fibrin and cellular elements was present toward the lumen. The recent thrombus was situated on a partly organized thrombus. In the latter some of the unorganized material had the appearance of fibrinoid, staining red with the trichrome method, and some of it was hyaline-like, staining partly red and partly blue. In PTAH only the red areas were bluish-purple, whereas the homogeneous bluish-red hyaline material appeared light brown. A thick layer of dense collagenous tissue was situated below the organizing thrombus, and in some sections small and large atheromata were found within the latter. The above-described dense collagenous tissue was not present in Case 1. A moderate number of capillaries could be demonstrated in this collagenous tissue.

The media was atrophic, thin, and fibrous. It contained numerous capillaries, venules, and arterioles. These vessels were surrounded by numerous plasma cells, lymphocytes, and histiocytes.

Comment

Arteriosclerotic plaques are generally regarded as the result of a reactive fibrosis following deposition of lipids. One hundred years ago von Rokitansky [6] expressed the view that arteriosclerotic lesions result from an "encrustation" due to a blood "dyscrasia." Recently Duguid [2,3] and Crawford and Levene [1] forwarded the idea that arteriosclerotic plaques represent mural thrombi which have been incorporated into the arterial wall and organized. Duguid, but particularly Crawford and Levene, regard atheroma as a result of degenerative changes taking place in such mural thrombi. More recently Weiss et al.[7] reported three cases of mural fibrin thrombosis of the descending aorta but expressed the view "that mural fibrin thrombosis can be identified in any of its stages of progressive organization as an entity apart from atherosclerosis."

In both the two cases that we studied, numerous white opaque pearly-white plaques covered the aortic intima. The white opaque plaques were identified microscopically as recent fibrin thrombi. The pearly-white plaques, on the other hand, were composed of a young, acid-mucopolysaccharide-rich connective tissue, containing capillaries, numerous young smooth muscle cells, and remnants of unorganized thrombus material. Other pearly-white plaques were composed almost entirely of mucopolysaccharide-rich tissue with no remnants of unorganized thrombus. Newly formed collagen and elastic fibers were present in the polysaccharide-rich tissue, mainly around capillaries and smooth muscle cells.

We believe that these various microscopic changes—recent fibrin thrombi; recent fibrin thrombi with some conversion into AMP; AMP-rich tissue with remnants of unorganized thrombi, and AMP-rich tissue with no remnants of thrombus—all represent stages in the organization of mural fibrin thrombi. According to this interpretation, the pearly-gray plaques represent older thrombi, completely converted into connective tissue, at one end of the scale, and the white opaque plaques represent thrombi composed almost exclusively of fibrin and cells, at the other end. The outcome of this process is the formation of a fibrous plaque, the origin of which is difficult to determine, when the steps in its development from recent thrombi are no longer demonstrable.

The two cases here reported do not permit final conclusion as to whether fibrous plaques, developing from fibrin thrombi, represent an uncommon lesion, or whether fibrin is one, or possibly the only, factor in the genesis of typical fibrous plaques seen in the common form of arteriosclerosis. The fact that fibrous plaques may arise in this fashion renders this problem worthy of further investigation.

Whether thrombi were superimposed on atheromata, or whether atheromata had

arisen within the thrombus, as suggested particularly by Crawford and Levene, also remains an open question. Some findings, namely, the presence of unorganized fibrinous material within atheromata, support the latter view. On the other hand, atheromata were present also within the diffusely thickened intima, i. e., within the fibrosis attributed to ageing.[4]

The focal nature of the lesions indicates that local factors did play an important role in the development of these mural thrombi. However, a blood "dyscrasia" in the broadest sense was present in both cases, both having had a terminal uremia, and, in the first case, a uremia of long duration. Uremia is known to cause fibrinous deposits on the surface of serous membranes. Fibrinous uremic pericarditis, pleurisy, and pneumonia were conspicuous findings in Case 1. Uremia might have had a similar effect on the aortic intima.

Summary

Two cases of marked arteriosclerosis of the aorta characterized by the presence of multiple pearly-white and white opaque plaques are described.

The white opaque plaques were shown to consist of recent thrombus material, predominantly fibrin. The pearly-white plaques were recognized as older thrombi in various stages of organization. Some still contained remnants of unorganized fibrinoid-like thrombus material. The organized parts consisted of acid-mucopolysaccharide-rich tissue, in which numerous smooth muscle cells, a moderate number of capillaries, and, depending on the stage of the organization, collagen and elastic fibers had developed.

There was some evidence that atheromata may arise within such organizing thrombi. However, this question, as well as the significance and frequency of mural fibrin thrombi in the genesis of common arteriosclerosis, remains an open question.

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Ovarian Transplants in Abdominally Placed Testes of Mice (Strain C57)

PAUL W. ELLIOTT, M.D., and ELIAS HUSNI, M.D., Indianapolis

Biskind and Biskind [1] first noted the development of ovarian tumors from intrasplenic transplants of ovarian tissue in castrated rats. Furth and Sobel [2] were successful in similar experiments with mice, while Lipschütz, Ponce de Leon, Woywood, and Gay [3] have had similar results with guinea pigs. Recently Gardner [4] transplanted ovarian tissue into several sites, including the testes of mice, which apparently remained within the scrotum. He reported the development of one testicular tumor which he attributed to a response of the interstitial cells of the testis. The remainder of his transplants showed only degenerative changes.

The purpose of our experiment was to study the growth of intratesticular ovarian transplants within the abdominal cavity. Thirteen Strain C57 black litter-mate mice, 8 weeks old, were used. With the mice under ether anesthesia and moderately sterile conditions, the ovaries were removed through a midline transperitoneal incision. Each ovary was then divided into two pieces and placed in an isotonic saline solution containing aqueous penicillin and dihydrostreptomycin. A male mouse was then anesthetized and both testes drawn into the abdominal cavity, using the same approach. One testis was excised. The ovarian tissue was transplanted by trocar into the remaining gonad. The testis was left in the abdominal cavity. Three days following this procedure the animals were given subcutaneously 0.3 ml. of human urine which was frog-positive with 0.1 ml. for pregnancy.

All of the animals were killed five months later, and growth of the transplants was found in five of the animals. The testes were in the abdominal cavity at the time of examination. Grossly, the lesions were 3 to 4 mm. in diameter and approximately spherical. There was one exception. This one had a 2 mm. nodule which was 3 mm. from the original transplant. Microscopic examination demonstrated this nodule to be located along the path of the trocar. The lesions were dark-red and well demarcated and caused a bulge in the tunical albuginea. The blood vessels of the tunical abuginea over the transplants were prominent.

Histologically the growth patterns were mature. The transplants compressed the surrounding seminiferous tubules, which were atrophic. Very little fibrous tissue response was present about the transplants. There were many large cyst-like spaces filled with pink-staining fluid and lined by several layers of granulosa cells. The granulosa cells formed nodules in some areas.

In other areas groups of cells had more vesicular nuclei with abundant, finely granular cytoplasm containing vacuoles. The cytoplasm of some of these larger lutein-like cells contained a brown finely granular pigment, and the nuclei of these cells tended to be pyknotic and hyperchromatic. The material within the cytoplasm gave a plus-minus reaction to acid-fast stain. The cells in several groups of this type were counted, and peripheral chromatin bodies in the nuclei were found to be present in 70% to 80% of the cells. Reticulum stains revealed numerous fine interlacing strands between the lutein-like cells but not within the granulosa cells. Two of the five transplants contained an ovum, and one had viable Fallopian tube within it.

Submitted for publication Dec. 7, 1956.

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Fig. 1.—Section through a margin of the transplant. Note atrophy of the seminiferous tubules adjacent to the transplant. The small cyst-like structure is surrounded by several layers of cells, each of which has a small, oval, or round, hyperchromatic nulceus surrounded by a scanty, eosinophilic cytoplasm. Above this are several columns of paralutein-like cells. The rest of the cells in the transplant are similar to the ones in Figure 2. Reduced to 87% of mag. × 130.

Fig. 2.—The cells have abundant, finely granular cytoplasm and many vacuoles. The nuclei are somewhat pyknotic, and the cytoplasmic nuclear ratio is high. Reduced to 87% of mag. × 800.

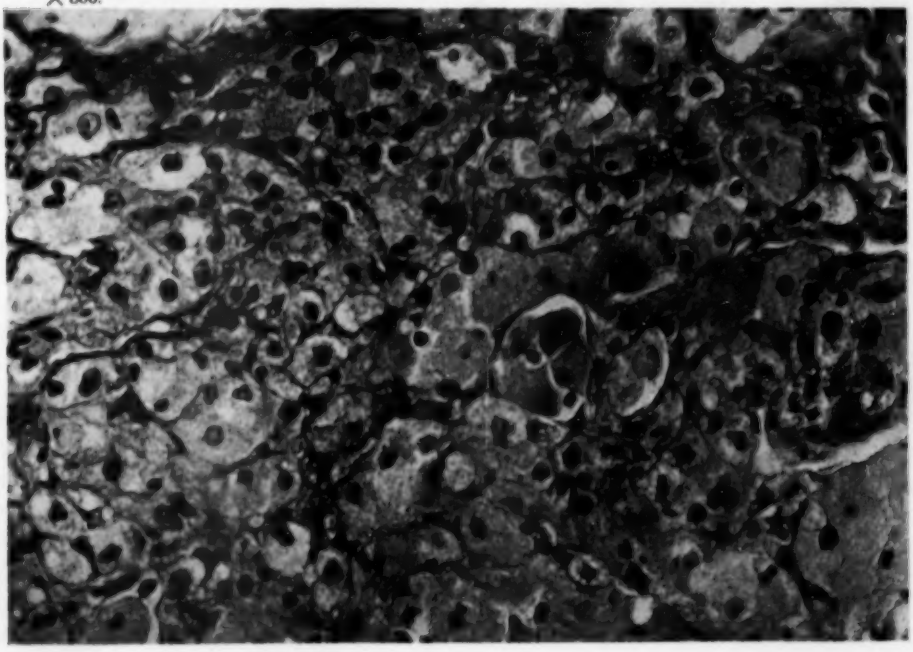




Fig. 3.—Note the atrophy of the seminiferous tubules in the upper left-hand corner and nodule of granulosa cells above the ovum. Reduced to 87% of mag. × 250.

Summary

The growth of ovarian transplants in abdominally placed testes in 5 of 13 C57 mice has been described. This growth was over a five-month period. Although by strict definition these transplants are tumors, we feel that hyperplasia of the transplant better described the process observed in this experiment.

We believe that the larger cells with abundant cytoplasm are lutein, and not interstitial cells of the testis. Eighty per cent of these cells have the peripheral chromatin body along the nuclear membrane, indicating that they are of female type.[5,6] Although these cells resemble morphologically the interstitial cells described as resulting from estrogen stimulation in the testis,[7,8] as well as lutein cells, their reaction to acid-fast stain is only plus-minus. This is an indication that these cells are not the result of an estrogen stimulation of interstitial cells, for the ceroid in the reactive interstitial cells has a 4+ reaction to acid-fast stain.[9]

756 N. Graham Ave.

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News and Comment

DEATHS

Dr. Frederick H. Lamb Dies.—Dr. Frederick H. Lamb, of Davenport, Iowa, died on Jan. 25, 1957.

GENERAL NEWS

Bertner Foundation Award.—The Bertner Foundation Award for 1957 from the University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, was presented to Dr. John J. Bittner, of the University of Minnesota Medical School, on March 8. In accepting the award, Dr. Bittner talked on "Studies on Mammary Cancer in Mice and Their Implications for the Human Problem."

Films of Possible Interest to Pathologists

Title: "An Aid to Therapy: Bacterial Antibiotic Susceptibility Testing"
28 minutes, color, sound, January, 1957
Authors: Drs. Leo L. Leveridge and Frederick C. Fink
Sponsor: Pfizer Laboratories, Brooklyn
Distribution: Pfizer Laboratories, Brooklyn

Title: "Stress and the Adaptation Syndrome"
35 minutes, color, sound, December, 1956
Authors: Dr. Hans Selye
Sponsor: Pfizer Laboratories
Distribution: Pfizer Laboratories, Brooklyn

Title: "Vitamin Deficiencies in Pregnancy" (revised American version)
34 minutes, color, sound, February, 1957
Authors: Drs. Norman P. Schenker, Hubert de Watteville, Rudolph Jurgens
Sponsor: Hoffmann-La Roche, Inc., and Medical and Scientific Films, Inc.
Distribution: Medical and Scientific Films, Inc., 330 E. 41st St., New York 17

Title: "Replacement Transfusion for Erythroblastosis Foetalis"
17 minutes, color, sound, 1956
Authors: Dr. Alice M. Goodfellow and Eleanor M. Atkins, R.N.
Sponsor: Mervin LaRue, Inc., for Baxter Laboratories, Inc.
Distribution: Baxter Laboratories, Inc., Morton Grove, Ill.

Title: "Disorders of the Heart Beat"
19 minutes, color, sound, November, 1956.
Authors: American Heart Association
Sponsor: Churchill-Wexler Film Productions, for Wyeth Laboratories, Inc.
Distribution: Wyeth Laboratories, Inc., Philadelphia

Title: "Microglia"
10 minutes, black and white, sound, 1956.
Authors: Dr. C. M. Pomerat with Walter Hild, E. E. Pitsinger, C. G. Lefeber
Sponsor: Wynne S. Eastman Laboratory, for Association of American Medical Colleges and Abbott Laboratories
Distribution: Association of American Medical Colleges and Abbott Laboratories

Title: "Oligodendroglia"
10 minutes, black and white, sound, 1956
Authors: Dr. C. M. Pomerat, Charles Lumsden, Curtis Haskins, C. G. Lefeber
Sponsor: Wynne S. Eastman Laboratory, for Association of American Medical Colleges and Abbott Laboratories
Distribution: Association of American Medical Colleges and Abbott Laboratories

Title: "Action of the Human Heart Valves"
24 minutes, color, sound, 1955

Authors: Drs. K. P. Klassen and C. V. Meckstroth
Sponsor: American Medical Association
Distribution: A. M. A. Film Library
Title: "Termination for the Bile Duct"
20 minutes, color, sound, 1956

Authors: Dr. Julian A. Sterling with Al Kane Productions
Sponsor: American Cyanamid Co.
Distribution: American Cyanamid Co., Danbury, Conn.
Title: "Electron Microscopy of Normal and Leukemic Leukocytes"
40 minutes, black and white, sound, 1955.

Author: Dr. Marcel C. Bessis, Paris, France
Sponsor: Association of American Medical Colleges
Distribution: Association of American Medical Colleges Film Library
Title: "Normal and Abnormal Platelets"
20 minutes, black and white, sound, 1955

Author: Dr. Marcel C. Bessis, Paris
Sponsor: E. R. Squibb & Sons
Distribution: E. R. Squibb & Sons, 745 Fifth Ave., New York 22

Information on prices of these films can be obtained from either the author or the sponsor.

Books

Clinical Chemistry: Principles and Procedures. By Joseph S. Annino. Price, $7.50. Pp. 280, with 15 figures, 11 tables, 2 charts. Little, Brown & Company, 34 Beacon St., Boston 6, 1956.

This book is designed to assist in the setting up and operation of a hospital chemistry laboratory. Its value in serving the supervisory and technical personnel will depend largely on the needs of existing laboratories for this type of text. The division of the book into two sections, (1) basic techniques and fundamental information, and (2) methods, allots about 25% of the space to a review of general principles and the remainder to specific methods of analysis. The author points out that certain basic traits of technicians are desirable, including interest, intellectual honesty, and a conscientious attitude. Recognizing the importance of fostering these qualities, he has made an effort to make this book interesting, as well as instructive, to the persons performing the tests. This has been done by inserting in each procedure a section describing the principal reactions and precautions involved in the operation and a second section discussing the physiological significance of the substance being determined. This book would be an asset to any clinical chemistry laboratory.

Adrenal Function in Infants and Children: A Symposium. Edited by Lytt I. Gardner, M.D. Price, $6.75. Pp. 221, with illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1956.

This book embodies material from a symposium on Adrenal Function in Infants and Children, held under the auspices of the department of pediatrics of the State University of New York. This symposium was made possible by financial support of the M & R Dietetic Laboratories, Inc., and the volume is edited by Lytt I. Gardner. Eighteen contributors interested in various aspects of adrenal function participated in the conference. The volume should be useful to physicians who care for children with diseases of the adrenal glands. The discussions of the papers which were presented at the conference are also included.

Current Therapy 1957. Edited by Howard F. Conn, M.D. Price, $11.00. Pp. 731. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

This edition is the ninth that has appeared since 1949. According to the authors, it represents an attempt to apply therapeutic recommendations which reflect newly acquired knowledge of the nature of disease processes. Each article is original and is meant to emphasize current ideas of therapy dealing with various phases of medical practice. The book is of an encyclopedic type, and obviously cannot be reviewed in detail. The large number of contributors and the able group of consultants testify to the thought that has been given to the revision of this volume. The book will be of great value to all physicians who wish to become familiar with the various ideas about therapeutic measures which are deemed to be improvements. Various tables supply readily available information about poisonous substances in household items, principal active ingredients in various commercial products, a roster of drugs, metric and apothecary systems, and normal laboratory values of clinical importance; an index of authors and of subjects is included. In short, an enormous amount of information of therapeutic interest is available in this well-printed and well-bound volume.

Progress in Nuclear Energy, Series VI, Biological Sciences. J. C. Bugher, J. Coursaget, and J. F. Loutit, Editors. Price, $7.00. Pp. 205. McGraw-Hill Book Company, Inc., 55 Fifth Ave., New York 3, 1956.

This book contains a somewhat heterogeneous collection of papers, ranging from the application of radioisotopes in studies of photosynthesis and mineral metabolism to an examination of the genetic and other hazards to man from ionizing radiations. Some of these papers are reviews, and others are summaries of review papers, presented at the First International Conference for the Peaceful Uses of Atomic Energy, held at Geneva in August, 1955. This book, the first in Series VI, is devoted to an examination of progress in the Biological Sciences, as the title indicates. As such, it may be of some value to specialists in the

fields that are touched upon in this volume, but its unevenness and lack of continuity with many gaps would not appear to make it a particularly attractive addition to the bookshelf of the general scientific reader who wishes to inform himself of progress in nuclear energy as applied to the biological sciences. As a reference work it will be of value.

Analytical Pathology. Edited by Robert C. Mellors, M.D., Ph.D. Price, $12.00. Pp. 477, with 118 illustrations. The Blakiston Division, McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1957.

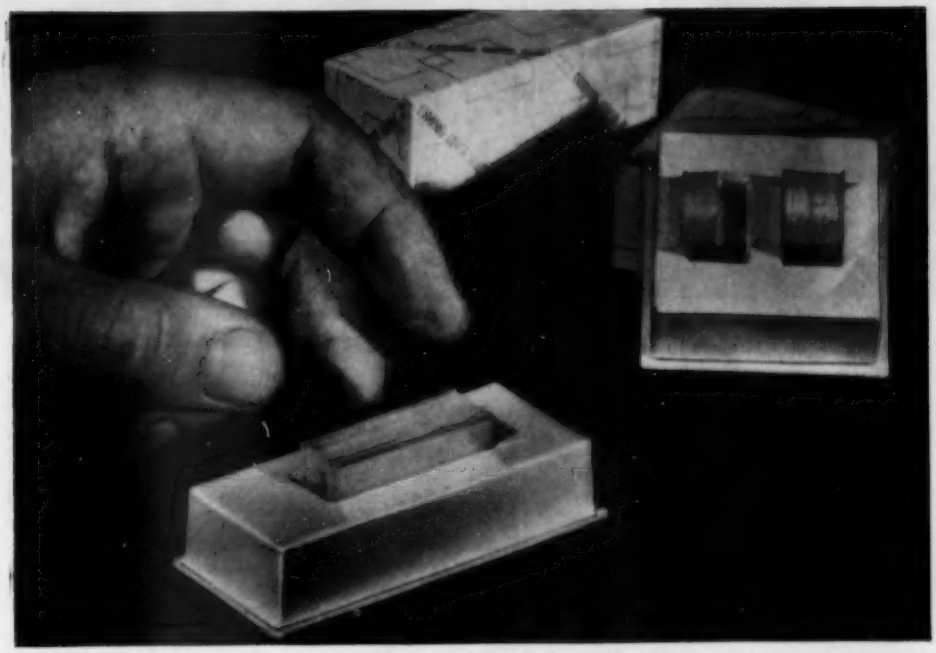
This book is a collection of seven review articles on Cancer, Arteriosclerosis and Hypertension, Inflammatory and Vascular Disease of the Glomerulus, Three Aspects of Hepatic Failure, The Adenohypophysis, the Macrocytic Anemias and Abnormal Hemoglobins, and The Role of Connective Tissue in Hypersensitivity Reactions. The authors, in order of appearance, are R. C. Mellors, J. W. Gofman, D. B. Jones, O. D. Ratnoff, A. B. Russfield, J. F. Mueller, R. W. Vilter, J. J. Will, and B. M. Wagner. The attempt has been made to present descriptive and experimental observations on the various subjects in the realms of morphology, physics, and chemistry. With some variation, the authors and editor have achieved this very well. From the informational standpoint the book is very interesting. The material is well presented. The tables, charts, and illustrations are excellent. For the modern pathologist not engaged in teaching this book offers information on a range of subjects now under active investigation. For the teacher of pathology this book offers collected information which can be used to supplement the more conventional morphologic texts. For the investigator in a particular area the individual sections represent one author's current view of the field. This is a useful book, though the price may limit its use to some extent.

Biochemistry of the Developing Nervous System, Proceedings of the First International Neurochemical Symposium. Heinrich Waelsch, Editor. Price, $11.50. Pp. 537. Academic Press Inc., 125 E. 23d St., New York 10, 1955.

This volume records the proceedings (including discussions of papers and bibliographies) of the highly successful First International Neurochemical Symposium, held at Magdalen College, Oxford, in July, 1954. Various private and governmental agencies sponsored a remarkable collection of leading scientists from Germany, Italy, Norway, the United Kingdom, the United States, the U. S. S. R., Canada, Sweden, Belgium, and Denmark. Numerous disciplines were represented, reflecting the widespread and accelerating interest in neurochemistry.

The book is divided into six parts: I. Morphological and Functional Ontogeny; II. Chemical Composition; III. Dynamic Biochemistry (blood-brain barrier, gas exchange, and intermediary metabolism; IV. Enzymatic Differentiation in Relation to Function; V. Cellular Chemistry; VI. Intrinsic and Extrinsic Factors in Development (genetics, hormones, metabolic lesions, and enzymatic inhibitors). Perhaps of special interest to pathologists are papers on quantitative histochemistry by Pope and Lowry and a discussion of neurolipoidosis by Klenk.

A second symposium has since been held, and a third is currently being planned.



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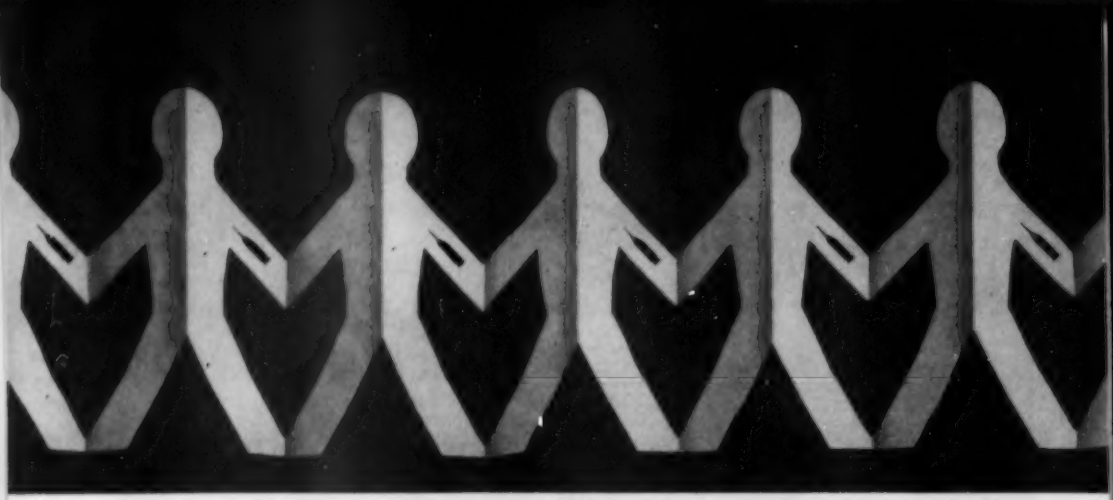
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The Test of Reliability. You can perform a simple test which will demonstrate the reliability and convenience of Diagnostic Plasma. First, take three vials of Diagnostic Plasma and run a prothrombin time test on each in the usual way. Then repeat, using three different vials of any other commercial control plasma, and three samples of pooled human plasma. The same thromboplastin preparation should be used in each test. See how Diagnostic Plasma consistently gives results identical with pooled fresh normal plasma, assuring valid interpretation of prothrombin times. We will be glad to send you three vials of Diagnostic Plasma to help you make this evaluation.

Diagnostic Plasma is also used in the coagulase test for the accurate identification of pathogenic staphylococci. It is available from laboratory supply distributors in boxes of 10 "no-waste" vials, $9.00.

Diagnostic Plasma

WARNER-CHILCOTT

Laboratory Supply Division

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☐ Without obligation on my part, please send me three vials of Diagnostic Plasma Warner-Chilcott.

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Paragon Tray Drawer Cabinet

Compact



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C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¼ x 4⅛

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C221—Capacity 1500 Slides—18¾ x 11 x 3⅛
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

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